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IMMUNITY AND SPECIFIC THERAPY





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# IMMUNITY AND SPECIFIC THERAPY

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## PREFACE

IN writing this book I have attempted to give a connected and symmetrical outline of the chief facts definitely known with regard to the method in which the body protects itself against infections, and of their applications in the diagnosis, prevention, and treatment of disease. It is not written in support of the views of any particular school of thought, and, when dealing with subjects still under discussion, I have tried to give a fair and impartial, though necessarily succinct, account of each of the rival theories. The factors in many of the problems of immunity are so complex, and our knowledge of the subject grows and alters so rapidly, that it is quite impossible to deal with it dogmatically at the present time. I have kept in view, as far as possible, the requirements of the physician and surgeon who may require an epitome of the theoretical basis of the modern methods of diagnosis and treatment, now assuming so much importance, and of the student who desires a general survey of the subject before commencing more advanced studies.

My best thanks are due to Mr. H. K. Lewis for the ready and courteous way in which he has acceded to all my suggestions and requirements; to Drs. Whitfield and Briscoe, from whom I have received some valuable suggestions; and to Professor Herbert Jackson, of King's College, for kindly reading the sections dealing with the more purely chemical and physical questions and for much useful information connected therewith. I have also to thank Sir Almroth Wright and Drs. R. W. Allen, Eyre, and Bolduan; Messrs. Macmillan and Co., Kegan Paul and Co., and the proprietors of the *Lancet*, *British Medical Journal*, and the *St. Bartholomew's Hospital Journal* for permission to use illustrations from their publications.





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## ERRATA

- Page 9, line 5 from bottom, omit "to" after "-cytes."
- „ 14, line 13, for "rather of" read "than with."
- „ 36, line 22, for "of" read "to."
- „ 45, line 29, for "antitoxin" read "toxin."
- „ 48, line 14, for "became" read "become"; line 25, for "united with" read "injured."
- „ 52, line 10, for "supernatural" read "supernatant."
- „ 55, line 4 from bottom, for "properties" read "effects."
- „ 57, line 1, for "is" read "are"; bottom line, insert "upon" after "toxins," and for "defends" read "depends."
- „ 70, line 6, for "hæmoglobin" read "hæmolysin."
- „ 78, lines 17 and 19, for "c.c." read "parts"; and line 18, for "16.6 c.c." read "16.6 parts."
- „ 79, top line, for "antitoxin" read "toxin."
- „ 90, line 22, for "toxin" read "antitoxin."
- „ 91, line 18, for "toxic" read "neutral"; line 26, omit "as."
- „ 93, line 22, for "injection" read "infection."
- „ 106, line 26, for "it" read "the toxin."
- „ 107, line 21, for "to tetanus antitoxin" read "of tetanus toxin."
- „ 110, line 9, for "antitoxin" read "toxin."
- „ 122, line 3, for "toxin" read "antitoxin."
- „ 128, line 20, for "leucocytes" read "bacteria ingested."
- „ 129, line 3 from bottom, for "toxin" read "antitoxin."
- „ 152, line 7, for "joined" read "formed."
- „ 171, line 10, for "rabbit" read "goat."
- „ 192, line 30, for "nephrotoxin" read "hepatotoxin."
- „ 220, line 7 from bottom, for "they" read "it."
- „ 252, line 12, for "leucocytes" read "bacteria."
- „ 256, line 15, for "complement" read "amboceptor."
- „ 284, line 13, for "opsonix" read "opsonic."
- „ 287, line 8, for "which in" read "in which."
- „ 290, line 19, for "bacteria can" read "leucocytes can."
- „ 296, line 8, for "bacteria" read "leucocytes."
- „ 307, line 12, for "local" read "general."
- „ 310, line 6 from bottom, for "y" read "a."
- „ 323, line 1, for "or" read "on"; line 28, for "complement" read "amboceptor."
- „ 345 line 13, should read, "those which have no defensive layer, or which have numerous receptors" etc.
- „ 348, line 14, for "bacteria" read "leucocytes."
- „ 351, line 14, for "installations" read "instillations."
- „ 355, last line, for "research" read "defence."
- „ 360, line 3, for "able" read "unable."
- „ 365, line 29, for "stable" read "labile."
- „ 378, line 8 from bottom, read "are accompanied by but the slightest," etc.
- „ 386, line 21, for "benefits" read "benefit."
- „ 407, line 10 from bottom, for "rise" read "use."
- „ 419, line 25, for "heated" read "beaten."



## GLOSSARY

- Active immunity.** Immunity due to an active struggle against some infective material, vaccine, or toxin.
- Addiment.** See Alexin or Complement.
- Agglutinin.** A specific antibody which brings about agglutination—*i.e.*, causes the bacteria, cells, etc., for which it is specific, to collect into clumps. Non-specific substances (acids, etc.) have a similar action, but are not properly termed agglutinins.
- Agglutigen.** The antigen of agglutinin—*i.e.*, the substance which, when injected into a suitable animal, leads to the formation of agglutinin.
- Agglutinoid.** A modification of agglutinin which has retained the power of uniting with the specific bacteria, etc., but has lost that of causing them to clump.
- Aggressin** (*aggredior*, I attack). A substance secreted by bacteria and possessing the power of inhibiting phagocytosis of the organism producing it.
- Alexin** (*ἀλέξω*, I ward off). A defensive substance having an injurious effect on bacteria, and occurring in the serum of normal and immune animals. It is analogous in many respects to the bacterial toxins, and, like them, easily destroyed by heat, chemical agents, etc. It is probably identical with complement, *q.v.* (For other synonyms, see p. 143.)
- Amboceptor** (*ambo*, both, and *capio*, I take). A specific antibody produced by the injection of bacteria, red corpuscles, cells, etc., and exerting, with the help of alexin or complement, a solvent action on these substances. The term is Ehrlich's, and its use should involve the acceptance of his theory of its action. (For synonyms, see p. 142.)
- Anaphylaxis** (*ἀ* or *ἀνα*, privative, and *φυλάσσω*, I guard). The opposite of prophylaxis—*i.e.*, a condition in which the susceptibility of the animal (especially to toxins, serums, etc.) is abnormally increased. Practically identical with hypersensitiveness.
- Antibody.** A substance formed by the injection into an animal of a substance (its antigen) not normally found in the juices of that animal (and probably in all cases of proteid constitution, or closely allied thereto), which unites with its antigen and modifies it in some way.
- Antiferment, or antienzyme.** An antibody to a ferment or enzyme.
- Antigen** (*ἀντι*, against, and *γίγνω*, I produce). A substance which, when injected into a suitable animal, has the power of leading to the production of an antibody. In most, if not in all, cases it is proteid in nature.
- Antitoxin.** An antibody to a toxin—*i.e.*, a specific substance formed by an animal in consequence of the presence in its tissues or juices of a given toxin, which the antitoxin thus produced has the power of neutralizing.
- Arthus' phenomenon.** A form of hypersensitiveness to serum, where in a sensitized animal local lesions (gargrene, abscesses, etc.) develop in a region where serum is injected, and the animal may become cachectic and die.
- Atrepsy** (*ἀ*, privative, and *τρέφω*, I nourish). A condition in which an infective agent or collection of malignant cells dies in the animal body owing to its being unable to obtain suitable nourishment: a conception introduced by Ehrlich to explain certain forms of immunity—*e.g.*, to malignant growths.
- Attenuation.** The change which an organism undergoes whereby it becomes less virulent.



**Autohæmolysin.** A substance which has the power of dissolving the red corpuscles of the individual which produces it; an autochthonous amboceptor or immune body to an animal's own red corpuscles.

**Bacteriolysis.** The phenomenon of solution of bacteria, more especially by the action of specific antibodies, aided by alexin or complement.

**Bacteriotropin, or bacteriotropic substance** (τρέπω, I turn). A substance (usually a specific antibody) which has the property of uniting with bacteria, and in some way altering their properties, usually rendering them more suitable for phagocytosis.

**Bordet's phenomenon.**—The absorption of alexin or complement which is brought about by means of a cell or bacterium combined with amboceptor or immune body, apart altogether from the alexin which is necessary for the action; the complete removal of all alexin from a fluid by means of a cell-immune-body compound.

**Chemotaxis** (τάξις, an arrangement). The attraction or repulsion of leucocytes, bacteria, etc., by substances in solution in the fluid containing the cells in question.

**Complement** (compleo, I fill up). A synonym for alexin, *q.v.* The term was introduced by Ehrlich, since on his theory this substance unites with the amboceptor which has already united with the bacterium, etc., and thus completes the conditions necessary for solution.

**Complementoid.** A modification of complement which possesses the combining powers of that substance, but which has lost its active solvent properties.

**Cytase.** Metchnikoff's term for the digestive (proteolytic) enzyme secreted by leucocytes; a synonym for alexin or complement.

**Cytolysin.** An antibody (immune body or amboceptor) to a cell, having the power of sensitizing that cell so that it is completely or partially dissolved on the addition of alexin.

**Cytolysis.** The solution—usually partial—of cells by means of an antibody (immune body or amboceptor) and alexin.

**Cytotoxin.** A substance acting as a cellular toxin, especially an antibody and alexin; practically identical with a cytolysin.

**Danysz effect.** The decrease in the neutralizing effect of antitoxin which is manifested when the toxin is added in portions, with an interval between each, rather than all at once.

**Desmon** (δεσμός, a bond). A synonym for immune body or amboceptor.

**Deviation of complement.** The phenomenon in which the solvent effect of immune body on cells or bacteria (in presence of alexin) diminishes as an excess of the antibody is added; also known as the Neisser-Wechsberg phenomenon.

**Dominant complement.** Where (on Ehrlich's theory) two or more different complements unite with a complex molecule of amboceptor which has united with a bacterium, corpuscle, or cell, that which has the more potent action is known as the dominant. Its effect may be produced without the action of the other complements, or the necessary amount may be smaller.

**Ehrlich's phenomenon.** The fact that the difference between the amount of toxin exactly neutralized by one unit of antitoxin and the amount which (added to one unit of antitoxin) just leaves one lethal unit free is greater than one lethal dose of simple toxin.

**Endocomplement.** A complement contained within a red corpuscle, probably in all cases lecithin or an allied substance.

**Endotoxin.** A bacterial toxin contained within the substance of the bacterium, and not liberated except when the cell is destroyed.

**Ergophore group** (ἐργον, work, and φέρω, I bear). The part of a molecule of antigen or antibody on which the specific properties of the substance depends (toxophore, zymophore, agglutinophore, etc.), in distinction from the haptophore or combining part of the molecule.

**Exotoxin.** A soluble bacterial toxin which is excreted by the bacterium into the surrounding fluid during the life of the organism.

**Fixation of complement.** A synonym for Bordet's phenomenon, *q.v.*

**Fixator.** A synonym for immune body or amboceptor.

**Gastrotoxin.** A cytotoxin or cytolyisin acting on the cells of the mucous membrane of the stomach.

**Gengou's reaction.** The removal of all alexin or complement from a fluid by means of a compound of a precipitin and its antigen; analogous with Bordet's phenomenon, except that in this case the reacting antigen is a soluble substance. The two are often grouped together as the Bordet-Gengou reaction.

**Group reaction.** A reaction with an antibody (usually an agglutinin) which is common to several species of bacteria, forming a well-defined group—*e.g.*, the coli group, or the pasteurelloses.

**Hæmagglutinin.** A substance which agglutinates red corpuscles.

**Hæmolysin.** A substance which dissolves red blood-corpuscles, or at least releases the hæmoglobin which they contain. The term is used mainly for an antibody having, in conjunction with alexin, a solvent action of this nature.

**Haptin.** A portion of a molecule of protoplasm having combining affinities for food molecules, and forming an antibody when shed (*v.* Receptor).

**Haptophore group, or Radicle** (*ῥαίζω*, I fasten). That portion of a substance (whether antigen or antibody) which has the power of entering into combination with its appropriate antibody or antigen, as the case may be. Thus a molecule of toxin is supposed to contain a group of atoms which can combine with a cell or molecule of antitoxin, and a second which can then exert a toxic action. The former is known as the haptophore group.

**Immune body** (*immunis*, exempt from public service). A specific antibody, produced by the injection of bacteria or other cells, and having the power of altering these substances in such a way as to render them completely or partially soluble on the addition of alexin. It is the same as amboceptor, but the term implies no theory and is generally preferable. Synonyms: substance sensibilatrice, desmon, preparator, copula, etc.

**Incitor constituent of serum.** A substance which aids phagocytosis, especially thermostable opsonin.

**Isoagglutinins.** An agglutinin which, occurring in the serum of a certain animal, will agglutinate the red corpuscles of other animals of that species, but not those of the individual which produces it.

**Isohæmolysin.** An immune body or amboceptor which, occurring in the serum of a certain animal, dissolves (in conjunction with alexin) the red corpuscles of other animals of that species, but not those of the individual which produces it.

**Koch's phenomenon.** The tuberculin reaction, or rise of temperature and sudden exacerbation of the local lesions occurring in a tuberculous animal after injection of a culture of tubercle bacilli, living or dead, tuberculin, or other specific tuberculous material.

**Lactoserum.** A serum containing a precipitin for milk proteids.

**Leucotoxin.** An antibody (immune body or amboceptor) which, in conjunction with alexin, exerts a toxic influence on leucocytes.

**Lysis** (*λύσις*, a loosening). The solution of cells, bacteria, etc., mostly by means of antibodies or other protective substances.

**L<sub>0</sub> dose of toxin.** The amount which is exactly neutralized by one unit of antitoxin.

**L<sub>+</sub> dose of toxin.** The amount which, added to one unit of antitoxin, behaves just like one lethal dose of toxin, bringing about a fatal result in test animals within the time-limit fixed. The fact that the L<sub>+</sub> dose—the L<sub>0</sub> dose is greater than one lethal dose constitutes the Ehrlich phenomenon.

**Macrocytase.** In Metchnikoff's phraseology, the digestive enzyme secreted by the large mononuclear leucocytes, and having a special action on cells rather than on bacteria; really a synonym for alexin, especially for one acting on cells or red corpuscles.

**Macrophage.** Metchnikoff's term for a large phagocyte which, according to him, is especially adapted to the ingestion of cells or corpuscles rather than of bacteria. They may be large lymphocytes, large hyaline cells, endothelial or other tissue cells.

**Microcytase.** The digestive enzyme of Metchnikoff's microcytes or polynuclear leucocytes; supposed to have a special action on bacteria. Practically identical with alexin.

**Microphage.** A small leucocyte supposed by Metchnikoff to be specially active against bacteria, and to have little or no phagocytic action on cells or corpuscles. They are polynuclear leucocytes.

**Negative phase.** The sudden diminution in the amount of an antibody (and possibly of other defensive substances) in the blood which follows immediately on the injection of an antigen.

**Neisser-Wechsberg phenomenon.** Deviation of the complement, *q.v.*

**Nephrotoxin.** A cytotoxin specific for renal cells.

**-ogen.** A suffix usually employed to denote an antigen in relation to its antibody—*e.g.*, agglutino-gen, the substance which on injection into an animal leads to the production of agglutinin. Also used for a preliminary non-active form of an active substance—*e.g.*, opsonino-gen, a substance which under certain conditions becomes opsonin.

**-oid** (*είδος*, a figure or appearance). A suffix denoting a secondary modification of an active substance in which it appears to retain its power of entering into combination with its antibody or antigen, but has lost its specific activity; a molecule of antigen or antibody which has lost its ergophore, but retained its toxophore, group—*e.g.*, complementoid or toxoid, *q.v.*

**Opsonin** (*opsono*—I cater for, I prepare for food. Derived from *δψον*, cooked meat, a sauce or relish). A substance or combination of substances of whatever nature which has the power of combining with a bacterium, cell, or other substance, and rendering it more easily ingested by a leucocyte or other phagocyte.

**Passive immunity.** Immunity due to the injection of serum from an animal which has acquired immunity to a toxin or infective agent.

**Pfeiffer's phenomenon.** The classical Pfeiffer's phenomenon consists in the globular transformation, loss of staining reaction, and finally complete disappearance of cholera vibrios, when introduced into the peritoneal cavity of an immunized guinea-pig, or into that of a normal one if immune serum be also injected. Also applied to the similar, but usually less complete, destruction of other bacteria under similar conditions, or to bacteriolysis in general.

**Phytotoxin.** A poisonous substance formed by one of the higher plants, but otherwise closely resembling a bacterial toxin, more especially in its power to give rise to the production of an antitoxin on injection—*e.g.*, ricin, abrin.

**Polyceptor.** Amboceptor which possesses several haptophore groups capable of anchoring several molecules of different sorts of complement, the most important of which is termed the dominant (Ehrlich).

**Polyvalent serum.** A serum containing antibodies against several strains of the same species of bacteria—*e.g.*, streptococci.

**Polyvalent vaccine.** A vaccine composed of the dead bodies of several strains of the same bacterial species. A vaccine composed of more than one species of organism is termed a *mixed vaccine*.

**Positive phase.** The period during which the amount of antibody or other protective body in the serum is increased owing to the injection of an antigen. In general terms it corresponds to the period of exalted im-

munity due to vaccination, injection of toxin, etc., and is very variable in duration.

**Precipitin.** An antibody to a soluble form of proteid, having the power of precipitating or coagulating that proteid by a process of clumping its molecules.

**Precipitogen.** The antigen to a given precipitin. Thus when a serum is injected into an animal numberless substances are introduced, a certain number of which only give rise to the formation of precipitin, and are called precipitogens. Also called precipitable substance.

**Precipitogenoid.** Heated precipitable substance, which has retained its power of combining with precipitin, but no longer forms a precipitate after doing so.

**Precipitoid.** Precipitin which has lost its active or ergophore, but retained its combining or haptophore, group; the latter has also increased in affinity for precipitable substance. The name is also applied to precipitogenoid.

**Predisposition.** The opposite of immunity; the state of an animal, in virtue of which it is readily infected with a given agent.

**Preparator.** Metchnikoff's term for immune body or amboceptor.

**Prophylaxis.** Any process by which the vulnerability of an animal by an infective agent or toxin is diminished or removed; a process for the induction of immunity, more especially in its practical application to the prevention of disease.

**Prostatotoxin.** A cytotoxin for the cells of the prostate.

**Pro-zone.** In constructing a curve indicating the action of an antibody at different dilutions, it sometimes happens that stronger solutions have less effect than more dilute ones. The region of the curve in which this inhibition of the action is brought about by an excess of the active substance is termed the pro-zone. It occurs with substances other than antibodies. Also called *zone of inhibition*.

**Receptor.** In Ehrlich's side-chain theory a part of a living molecule of protoplasm which has the power of attracting and combining with a molecule of food proteid (or of toxin, etc.) from the fluid with which it is bathed, and of building it up into the whole molecule, and thus utilizing it as nourishment, to aid which process it may also seize one or more molecules of complement. When shed into the blood these receptors constitute antibodies.

1. *Simple* (e.g., those constituting antitoxin). In the antibodies formed by this group we can only distinguish one group of atoms—a haptophore group having the power of combining with the specific antigen (e.g., toxin), and preventing its subsequent union with a living cell, thus rendering it inert.

2. *Complex* (e.g., agglutinin), in which we can recognize two separate properties, presumably situate in different groups of atoms: (a) a haptophore, combining group, as above; and (b) an ergophore group, on which the activity depends, and which may be destroyed whilst (a) remains intact.

3. *Compound* (e.g., amboceptor, on Ehrlich's theory). In them there are two or more haptophore groups, one of which combines with the antigen, the others with one or more molecules of complement.

**Sensitization** of bacteria, corpuscles, etc. The addition of immune body, so that the objects are prepared or sensitized to the action of alexin.

**Side-chain theory.** The theory (Ehrlich's) which accounts for the development of antibodies by supposing that the receptors (*q.v.*) which combine with the specific antigen may, under certain circumstances, be produced in excess and cast off into the surrounding fluid; these receptors, retaining their power of combining with antigen, constitute the antibodies in question. A brilliant conception, which has been the cause of enormous advance in our knowledge of problems connected with immunity.

- Smith's (Theobald) phenomenon.** The acquisition of hypersensitiveness to serum and other proteid substances (normally inert) which occurs in some animals as a result of minute doses of these substances, and leads to rapid death, with acute symptoms, when a second injection is given.
- Specificity** (*species*, an image). A direct relation of cause and effect between two substances (such as diphtheria toxin and its antitoxin, the latter being only produced by, and acting only on, the former), or between a substance and a phenomenon (such as the tuberculin reaction, produced only by tuberculous products in a tuberculous animal). The specific products of a micro-organism are those produced only by that organism, so that their recognition is proof of its presence. In the same way a specific disease is one produced only by a certain bacterium (such as diphtheria or anthrax), and not by several organisms (such as suppuration or actinomycosis).
- Spermatotoxin.** A cytolytic to spermatozoa.
- Stimulin.** A substance having the power of stimulating the action of the leucocytes (more especially in regard to phagocytosis) by a direct action on the leucocyte itself. The existence of these substances is doubtful, most of the phenomena supposed to be caused by them being due (*a*) to the action of opsonins, and (*b*) to substances which have a positive chemotactic action, attracting leucocytes to the region.
- Syngytiotoxin.** A cytolytic acting on the cells of the placenta.
- Thermolabile.** Easily destroyed by heat. In general thermolabile substances are destroyed, completely or partially, by an exposure to 55° C. for half an hour or to 60° C. for 10 minutes.
- Thermostable.** The opposite to thermolabile, *q.v.*
- Thyrototoxin.** A cytolytic acting on the cells of the thyroid gland.
- Toxin** (τοξικὸν φάρμακον, the drug with which poisoned arrows were anointed. τόξον, a bow). The specific poison on which the pathogenic activity of a micro-organism depends. The fact of its being specific excludes simple chemical substances which may also exert a toxic action.
- Toxoid.** A secondary modification of a toxin which has lost its power of producing toxic symptoms, but retained that of combining with antitoxin or susceptible cells; or, in Ehrlich's terminology, one that has lost its toxophore, but retained its haptophore, group.
- Toxone.** A specific substance of feeble toxicity and slight affinity for antitoxin which is supposed to be produced by certain bacteria, notably that of diphtheria, in which case it is believed to be the cause of paralysis. Unlike toxoid, it is a primary product. Its existence is doubted, and the effects attributed to minute amounts of toxin by some authors.
- Toxophore group.** The portion of a molecule of toxin on which the toxic activity depends, the destruction of which converts the molecule into one of toxoid.
- Trichotoxin.** A specific cytotoxin for ciliated epithelium.
- Vaccination.** The production of active immunity by some process less severe than the induction of an ordinary attack of the disease in question.
- Vaccine.** A substance (usually a dead culture or living culture of mitigated virulence) the injection of which leads to the production of active immunity with less risk than that which accompanies an ordinary attack of the disease.
- Virulence** (*virus*, a poison). The property or properties of an organism in virtue of which it is able to give rise to disease in animals or to produce a powerful toxin.
- Zootoxin.** A poisonous substance of animal origin which resembles in other respects (and especially in that it can give rise to the production of an antitoxin) the bacterial toxins—*e.g.*, snake venom, eel serum.
- Zymophore group** (ζύμη, leaven). The portion of an enzyme or enzyme-like substance on which the specific properties depend, in contradistinction to the combining or haptophore portion.



# IMMUNITY AND SPECIFIC THERAPY

## CHAPTER I

### INTRODUCTORY AND GENERAL

IMMUNITY is the power which certain living organisms possess of resisting influences which are deleterious to others. In its widest form it includes the power of resisting poisons, adverse physical influences, and diseases of all kinds. Thus, many men can and do acquire some degree of immunity against nicotine, alcohol, and other poisons; some bacteria are immune to temperatures which are quickly fatal to others; and some individuals and races have a very real immunity to gout and other metabolic diseases to which their less fortunate brethren are more prone. In any complete discussion of the subject these forms of immunity would require some consideration, but in what follows we shall, in the main, limit ourselves to the investigation of immunity against the diseases of bacterial origin. In doing so we must not be thought to consider the other diseases—metabolic and what not—as being unimportant. The very reverse is the case, and the subject which calls most urgently for research at the present day is the nature and mechanism of immunity against malignant tumours, and of this we have recently acquired a little knowledge. But the diseases other than those of bacterial origin will not be dealt with, for the simple reason that our knowledge of their intimate causes is still unknown, and until they are discovered, and until the physiological disturbances of the economy which occur in these diseases are more fully known, the nature of the corresponding immunity is obviously extremely difficult of study. The bacterial diseases are quite different, for here

the causes are fully known; the diseases themselves can be reproduced (in most cases) at pleasure, and the physiological disturbances which take place are fairly well investigated. There are, of course, gaps, and those not inconsiderable, in our knowledge; but, on the whole, the nature of these diseases is nearly as well ascertained as the present state of normal physiology will allow. Further, we can not only reproduce the diseases, but we can reproduce in most cases any degree of immunity to them which we may require for purposes of protection or research, and we can investigate the differences between the cells and fluids of the immunized person or animal and the corresponding parts of a normal organism, and we can attempt to correlate them with the production of the immune state. We have, therefore, a very large amount of information on the subject, and although this information is at present incomplete, we have already obtained results of the highest practical and theoretical importance; and the value of these results leads us to believe with confidence that our methods are right, that we are on the right track, and that a solution of the problems that have at present baffled research will come in the near future.

As defined above, immunity is a function of all living material, and one of the highest importance. Biologists have compiled lists of the essential properties of living protoplasm—nutrition, reproduction, and the like—but have not realized that immunity to bacterial action is the first necessity for continued life. Consider for a moment a small water animal—say a hydra—occurring in water which naturally contains saprophytic bacteria. Whilst the animal lives these organisms do not affect its protoplasm in any way, the latter being immune to their action; but on the animal's death rapid putrefaction occurs, and in a few hours its protoplasm is broken down by bacterial action: the immunity has ceased. Immunity to putrefactive bacteria is therefore a condition of life in the lower animals. But the same is true in every respect for those of a higher grade, man included. From the moment of birth we are surrounded with air containing bacteria which are not pathogenic in the ordinary sense, but which only fail to be so because of the inherent power of immunity to saprophytic bacteria, which is a fundamental property of all living material. Apart from this, the organisms present in the air, alimentary canal, skin, etc., would flourish as rapidly as they do in a corpse, and life would only be possible for a few hours,

or perhaps minutes. Readers of one of Mr. Wells's ingenious romances may perhaps remember how the strange beasts from Mars which invaded this planet died rapidly, being evolved in a region in which there were no bacteria, and in which this power of resisting their action had not been developed. The example is a striking one, and is strictly scientific, though we may wonder how the rotation of nitrogen, in which bacteria play so essential a part, is brought about in Mars; for this process of the breaking down of dead proteids by bacterial action, and the preparation of its nitrogen for use in plants, is essential for continued life on the planet. Without decomposition all the combined nitrogen of the world would soon become locked up in the dead bodies of animals; plants would starve and die, and animals (which are all dependent, directly or indirectly, on plant nitrogen) would likewise become extinct. It is a most marvellous natural phenomenon that these putrefactive bacteria should be found wherever life occurs, and wherever their aid may be required to deal with the protoplasm when dead, and that this same protoplasm should have acquired such potency in resisting their attacks whilst still alive. Absence of bacteria or absence of immunity are alike incompatible with animal life.

Considerations of this nature lead us to a short discussion of the difference between the pathogenic and non-pathogenic bacteria, and we find that there is, theoretically, none. Any bacterium will produce disease if it grows in the tissues of the living body, and all bacteria<sup>1</sup> will do so if the necessary degree and form of immunity is not present. A pathogenic organism is one which can grow in the living tissues, and it can do so only because those mechanisms of immunity which are sufficient in the case of the saprophytic bacteria are powerless to resist it; but in most cases, as we shall show, a higher degree of immunity can be produced artificially, and the microbe in question then becomes non-pathogenic to that particular animal. So, too, with the bacteria ordinarily regarded as non-pathogenic. Under certain circumstances, some of which are known and some still unknown, the resistance of the body or of a part of it may be broken down to such an extent that these organisms may gain access, flourish, and give rise to disease. Thus, *B. proteus* may give rise to phlebitis, growing in the thrombosed vein, and giving off toxins which have an injurious action on the tissues.

<sup>1</sup> Bacteria growing only at very high or very low temperatures, or on media very poor in nitrogen, perhaps excepted.

As a matter of high theory, therefore, there is no fundamental distinction between pathogenic and non-pathogenic bacteria, and we can imagine circumstances in which the tissues are vulnerable to attack by almost any microbic species. Practically, however, we shall consider an organism as pathogenic when the immunity of the animal which it attacks is not so perfectly developed that its presence in the tissues is but transient and unaccompanied by any noticeable ill-effects, but in which there is a balanced contest of longer or shorter duration between the injurious powers of the microbe and the defensive mechanism of the host, accompanied by more or less injury to the tissues and disturbances of the physiological economy of the latter, and resulting either in the death of the invader or of the patient. All grades occur. In most staphylococcic infections the chances are enormously on the side of the host, and the immunity is sufficiently high to localize the process before it has gone far. In typhoid fever the natural immunity and the pathogenic power of the organisms are more nicely matched; the contest between them is of long duration and doubtful issue. And in some forms of human disease, but more especially in artificial infections of animals with highly virulent cultures, the power of immunity seems almost nothing, the bacterium growing apparently unchecked and death occurring within a few hours. We say that these organisms have different degrees of pathogenicity, but it would be equally correct to say that there are different degrees of resistance against them, since an organism that is highly virulent towards one animal species may be quite harmless to another, so that pathogenicity is not an inherent property of certain bacteria.

Thus far we have considered the resistance of the host as if it were fixed and definite, but this is not the case. It has been known from time immemorial that certain diseases—especially those due to infection—are followed by a greater or smaller degree of immunity, so that a second attack is unlikely—at any rate, for some time. Smallpox, scarlet fever, and measles are amongst the most striking examples, and in them the protection given by the disease is in most instances absolute and lifelong. This is known as acquired immunity, and we shall enunciate it as a law that all recovery from infective disease is due to, and followed by, some degree of acquired immunity, though this may be slight, transient, and perhaps local.

Take, for example, a case of pneumonia, a disease which may

occur repeatedly and at short intervals in the same person. Pneumococci are widely distributed, and are almost universally present in the mouth; the necessary exciting cause, therefore, is always at hand. Under ordinary circumstances the power of resistance is sufficient to ward off the infection, but when this barrier of immunity is broken down by certain adverse circumstances—by excessive fatigue or starvation, by cold, or by an overdose of alcohol or other poison—the pneumococcus gains access to the tissues, and infection<sup>1</sup> occurs. The balanced contest spoken of above then takes place. The pneumococcus grows in the lungs and blood and produces a toxin, which tends to reduce the general health and the resistance of the body still further; and looking at the problem only from this side, it would appear that the process would go on until all the immunity was broken down, and the pneumococcus could flourish unchecked. This, indeed, might perhaps happen did not death supervene and bring with it conditions unfavourable for the growth of this organism. But all this time the tissues of the host have been reacting, and (in non-fatal cases) sooner or later a condition is brought about in which the noxious power of the coccus and the immunity of the patient are exactly level, so that the disease neither advances nor retrocedes; and the process goes still farther, and the patient develops such a degree of resistance as will not only render him immune to the spread of the infection, but will suffice to sterilize his tissues of the pneumococci which have already gained access. In other words, there has been an acquisition of immunity; the patient has become immune to the pneumococcus, and it is this, and this only, which has brought about the cure of the disease.

This process may be represented very diagrammatically, as shown on p. 6.

The line *ag* represents the degree of immunity to the organism in question, the pneumococcus. At *b* some event takes place (*e.g.*, exposure to cold) by which the resistance is lowered to such a degree that infection can occur. This takes place at *c*, with the result that the immunity falls still farther. At this time the bacteria begin to flourish in the tissues in increasing numbers. This is represented by the ascending line *i*. The immunity falls and bacterial action increases until a certain point is reached,

<sup>1</sup> I have elsewhere defined infection as the access of living, virulent, pathogenic bacteria to a region whence their toxins may act on the tissues of the body (Rose and Carless's "Surgery," sixth edition *et seq.*, chap. i.).

when the reserve forces of the patient have been brought into action, with the result that the immunity rises (from *d* to *e*). Somewhere during this rise (not necessarily or probably at its commencement) the contest turns in favour of the host; the bacteria are rapidly destroyed, and the disease is cured. Usually, but not necessarily, there is a rise to a level higher than the previous normal one (*e* to *f*), of longer or shorter duration, and then a reversion to the normal *g*. If exposure to cold again takes place, a fresh infection may now occur.

Now it must be emphasized that natural recovery from disease *only* takes place in virtue of an acquisition of immunity to the infecting agent, and in no other way; and, further, that, except in a few instances, medical treatment simply aims in aiding this phenomenon. If we exclude the various sera and vaccines, there

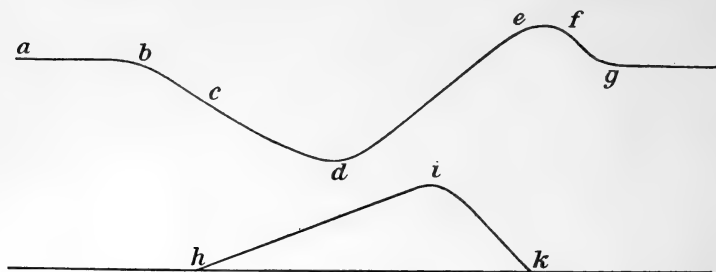


FIG. 1.

are but two therapeutic agents which have a direct curative effect—mercury in syphilis and quinine in malaria.<sup>1</sup> In these diseases the physician can apply a direct remedy, but in other cases the aim and object of treatment is to support the patient's strength until the natural development of acquired immunity takes place, and in some cases to aid this development by certain empirical means. It is found that all agents which tend to improve the general vitality and facilitate the performance of the normal physiological processes have this action; hence the importance of suitable food in amounts and at intervals suited to the patient's complaint, of fresh air at a proper temperature, of the removal of pain, and other symptoms which tend to impair the patient's

<sup>1</sup> Arsenic and some other drugs in the treatment of various protozoal infections (trypanosomiasis, etc.) may also be included. It is interesting to notice that all the diseases directly combated by simple means are protozoal in origin.

strength. These agents are all-important in medical treatment, but in themselves they are useless, and they only act by hastening the evolution of the immunity, without which the disease must necessarily progress to a fatal issue. This is well seen in the few diseases in which the development of immunity, in face of a natural infection, is but slight, or perhaps altogether absent, such as leprosy or hydrophobia. Here ordinary medical treatment is powerless, and all our hopes for the future are concerned with the discovery of a direct specific remedy.

It is this connection between immunity and recovery that renders the subject so important to the physician, and the neglect with which its study is treated by the general members of the profession a matter of such profound regret. In our medical education at the present day we pay, and rightly, much attention to the study of physiology, for without a knowledge of the processes of the healthy body we can hardly hope to diagnose and treat its derangements when diseased; and our physicians are in many cases competent physiologists. But it is equally important to understand the method in which the diseased body combats and cures an infection; and, although our knowledge of this is as yet imperfect, it is increasing day by day, and results of the greatest interest to the practising physician have already been obtained. And I, for one, think that an intelligent appreciation of what is actually taking place in the body, of the conservative and adverse forces, and of the conditions necessary for cure, will always be of value to the therapist, although it may not give any definite information as to what drug is to be prescribed.

Let us revert to the subject of NATURAL IMMUNITY. We may define it roughly as the immunity possessed by a certain individual in virtue of its belonging to a given animal species; it is inherent to a greater or less extent in all members of that species, and is not dependent on any event taking place during the life of the animal in question. In most cases it is present at birth, though this is not absolutely essential.

Examples are numerous. The lower animals are immune to the gonococcus, and, with few exceptions (the higher apes), to syphilis also. On the other hand, most of the diseases of the lower animals do not affect man—fowl cholera, canine distemper, and rinderpest are a few of many examples. In some cases all animals, with a few exceptions, are immune: this is the case with the venereal diseases, and in some of the protozoal infections of

the lower animals. In others different types of the infecting organism occur, and a given species is susceptible to one, immune to others; for example, there are three, and perhaps more, varieties of tubercle bacillus, which resemble one another in many points, and which attack respectively man, cattle, and birds, and each animal species is more or less immune to bacilli from animals far removed in the scale.

In general terms, the immunity or susceptibility of different animals depends to some extent on their zoological affinities. Thus man is pre-eminently susceptible to the *Spirochæta pallida*, the anthropoid apes less so, but still not immune, and the lower animals entirely refractory. Rinderpest affects cattle, sheep, goats, and other ruminants, and South African horse-sickness horses, asses, and mules. But to this rule there are numerous exceptions: thus, almost all warm-blooded animals are susceptible to anthrax, but the Algerian sheep and white rat are relatively immune, the wild rat being susceptible. And of the domestic animals we find cattle to be highly susceptible to tubercle, whereas goats, though closely allied zoologically, are almost immune.

Natural immunity does not exist to an equal degree in all individuals of a species. This is well seen in man during an epidemic, where, of a certain number of persons who are exposed to an infection (and, as far as we know, receive the same dose of the *materies morbi*), some escape the disease altogether, some have a slight, and others a severe, attack, whilst yet others die rapidly. Sex has some influence here, but it is usually difficult to trace, since the males and females of a community are in most cases exposed to an infection in varying degree.

Age is of more importance, and, in quite general terms, we may say that the younger the infant the less its immunity. Certain diseases, such as measles, scarlet fever, and whooping-cough, are rarely seen except in infants, and this is not altogether due to acquired immunity preventing a second attack in later life. Epidemic diarrhœa due to bacilli of the dysentery group is rarely seen—in this country, at least—except in the early years of life, and the same is true of cerebro-spinal meningitis and some other diseases. It is also interesting to notice that the variation in immunity may take a qualitative rather than a quantitative form. The best example is in the case of the pneumococcus. This organism is the chief cause of suppurative processes of whatever region in infants, whereas in adults it is (except in



certain regions) a decidedly rare cause of abscesses and other pyogenic processes. It is evident that the form of immunity which prevents the pneumococcus from gaining access to the tissues and giving rise to abscess formation is in abeyance in the young and well developed in the adult; yet the two are more nearly equal in their resistance to this organism in its rôle of a producer of pneumonia. There are also very marked differences in regard to local immunity in the two ages, but of those we shall speak subsequently.

Natural immunity must not be regarded as a fixed and definite quantity, since all individuals vary enormously in their resisting powers against various diseases at different times and under different conditions. The factors which tend to break down the immunity against any or all infections may be referred to as the *banal* causes of the diseases in question. They are not in themselves sufficient to lead to these diseases, but when they come into action *and an infecting agent is present* the disease will arise. Hence they are often referred to as predisposing causes of disease, and to the lay public they are the actual causes, since they are usually open and obvious, and the real infecting agent is, of course, unknown. They are of the utmost importance in preventive medicine, and wherever the probability of an infection is apprehended, a study of the patient's surroundings and habits may often lead to the giving of advice by which these banal causes of infection may be avoided and the disease warded off. In general these predisposing causes are a study for the physician rather than for the pathologist, and in some cases we are quite in the dark as to the method in which they act. Their study cannot be conveniently undertaken here before the mechanisms and processes of immunity have been described, but it will be useful to enumerate some of the more important.

Of these *cold* and *wet*, especially in combination, are unquestionably the most important. The exact way in which they act is not definitely known, but there are materials for a number of suggestions. Thus, as we shall have abundant opportunity of seeing, immunity is to a very large extent a function of the leucocytes, to which are specialized cells to which the defence of the body is entrusted. Now the functions (movement and phagocytosis) which can be easily investigated are found to be dependent in a very high degree on temperature, acting best at the temperature of the body, or slightly above; and it is highly probable that

the more subtle functions of the leucocytes may be similarly depressed by a low temperature. The exposure of the skin to cold, especially if the animal heat be abstracted more quickly by evaporation of moisture on the surface, will lead to a cooling of the blood which circulates through it, and hence to a slight, though appreciable, cooling of the whole blood. This, it is true, is soon compensated for, and no great amount of cooling of the whole body occurs; but even so, it is quite possible that the periodical chilling of the leucocytes during their repeated passages through the cold skin may be sufficient to diminish greatly their functional activity, and to lower the resistance to a point at which infection can occur, and when once pathogenic bacteria have gained a foothold, the resistance will for a time tend to decrease. There is also some evidence going to show that exposure to cold may lessen the production of the defensive substances which occur in the blood (alexin, antibodies, etc.), though this is not fully proved. It is worthy of note that the loss of immunity due to the action of cold and wet on one part of the body (such as the feet) is a *general* one, and may result in a nasal catarrh, an attack of pneumonia, acute rheumatism, etc., according to the nature of the infection at hand. It is not necessarily a local infection of the chilled region. This is very well shown experimentally. Fowls are immune to anthrax, but are rendered susceptible if they are kept for some time standing in cold water; and this acquired susceptibility is then a general one, and not merely of the feet.

Cold and wet, as is well known, have less action when accompanied by energetic muscular exercise, so long as this does not reach the extent of undue fatigue. This is not because less heat is lost during exercise. The reverse is the case. The suggested explanation is that the muscular metabolism leads to an increased production of heat, and at the same time the cutaneous capillaries are dilated and the heart accelerated, or that the circulation of blood through the skin occurs quickly; further, the internal temperature of the body may actually be raised several degrees. The result is that the temperature of any given leucocyte never falls much below normal, if at all, since it comes from the internal regions where the temperature is raised, passes rapidly through the skin, and returns again to the interior of the body.

The effect of *fatigue*, either alone or in conjunction with cold and wet, is also well known, and is one reason for the excessive mortality from disease of armies in the field. It is less explicable,

but may probably be connected in some way with the presence in the blood of katabolic products of muscular activity, which have an injurious action on the cells of the tissues in general and on the leucocytes in particular. Further, the metabolic products formed during the action of the muscles are acid in reaction, and it is found that some at least of the protective substances which occur in the blood (alexins and opsonins) act best in an alkaline medium. This diminution of immunity after muscular fatigue is manifested in animals as well as in man. White rats which have been made to work in a revolving cage are more susceptible to anthrax than normal white rats, the pre-existing immunity being broken down.

*Insufficient or unsuitable food* is a factor of importance, especially, perhaps, in the ætiology of tuberculosis. It is, however, rarely seen alone—in this country, at any rate—and in the poorer classes its effects are usually complicated by insufficient clothing, uncleanly habits, and by insufficient ventilation of their houses. For this reason we may perhaps be led to exaggerate its importance; and whilst it is, of course, true that semi-starvation, in common with other weakening influences, does pave the way for infective processes, we do not find that a supply of food restricted enough to cause a marked reduction of the bodily strength and some degree of anæmia is necessarily associated with any infective disease, though the patient may live under conditions in which infective material is present in abundance. This is well seen in fasting men, in hysterical anorexia, and in patients with impermeable œsophageal strictures. The blood, it may be pointed out, is not one of the tissues that suffers first in starvation, and its importance to the body in many ways is so great that it is kept in good functional activity whilst other regions waste quickly.

It is probable that insufficient food lowers the resistance of the body in certain directions rather than in others. In the East plague follows famine with some regularity, but there is little or no connection between famine and cholera. But in these latitudes at the present time the disease most commonly due to bad or insufficient food is tuberculosis. Formerly it was relapsing fever, or, as it was sometimes called, famine fever, a disease which is now almost extinct as a result of the general cheapening of foodstuffs.

It is worthy of note that the number of leucocytes per cubic centimetre diminishes in starvation, and is generally lower in the badly-nourished than in the well-fed; and these cells, as we shall see, are pre-eminently concerned in immunity, and this in a great

many ways. It was recognized long ago that post-mortem wounds are much more dangerous when received whilst fasting than during the process of digestion, and it is possible that this may be due to some extent to the increased number of leucocytes which occur in the blood during the process.

Exposure to a *vitiated atmosphere*, if of long duration, is a most potent cause of the breaking down of immunity, and when considered on a large scale, and in view of its effect on the general death and disease rate, is probably of greater importance than all other causes combined. It is especially important in connection with tuberculosis, and nothing is more striking than to notice its effect on the peasantry of some regions, in which, in spite of exposure to abundant fresh air during the daytime, and a supply of food which certainly does not fall below the physiological minimum, and is usually more abundant, phthisis and other tuberculous diseases are rife. These affections are in general common in cold and windy climates, and less prevalent in warmer countries, and there is little doubt that the main reason for this is the habit which dwellers in cold countries frequently contract of hermetically sealing all entrances to their rooms to keep out the cold. But this is frequently seen in warmer regions, and even throughout the South of England there is an almost universal opinion amongst the lower classes that night air is injurious. This is probably a survival from the time when malaria was indigenous in this country.

Apart from tubercle, the effect of bad air is especially manifested in the causation of diseases of the lungs, nose, throat, etc., and its effect is probably partly general and partly local. The effect of irritating vapours is, of course, local. Thus exposure to nitrous fumes is often followed by the rapid development of pneumonia, and this is, or may be, due to the pneumococcus, which is able to invade the injured lung.

We do not know the mechanism by which ordinary vitiated air acts on the general immunity.

Prolonged *anæsthesia* is probably a cause of considerable importance, though one not easy to estimate. The prevalence of ether-pneumonia is not yet ascertained, and has been hotly debated. It falls, of course, into the same category as the pneumonia due to irritating vapours, as described above. Apart from this, however, there is reason to believe that prolonged anæsthesia has some effect in lowering the general resisting

power of the body to the common pyogenic bacteria, and that the mere length of an operation should be an indication for the most scrupulous care in antiseptic precautions. It is perhaps conceivable that the anæsthetic drug present in the blood may be sufficient to paralyze the leucocytes for a sufficient time to allow bacteria to gain a foothold in the body.

Certain drugs, of which the most important is *alcohol*, have an important action in this respect. The liability of alcoholic subjects to pneumonia and some other infective diseases is well known, and in them the prognosis is more than usually unfavourable. We have but little knowledge of the action of alcohol in this respect. It may be that it acts as a direct inhibitor of the activity of the leucocytes, and it is known to destroy certain delicate defensive substances (alexins and opsonins) which play some part in the defence of the body against microbic invasion, but it is not known whether these effects are actually manifested in the circulating blood. It is also possible that alcohol tends to inhibit the formation of these defensive substances.

Alcohol tends to lower the temperature of the body by increasing the amount of heat lost. It dilates the superficial vessels and accelerates the heart's action in a way somewhat similar to muscular exercise, but does not, like it, raise the temperature of the interior of the body. Hence the effect of alcohol in conjunction with cold and wet is to increase their ill-effects. More blood is forced through the chilled skin and more heat is lost. The injurious effect of alcohol during exposure to cold is well known. The results, however, are different when alcohol is taken after exposure, and when the sufferer has reached warmth and shelter. There the increased flow in the cutaneous capillaries leads to a warming of the skin and consequent cessation of the chilling of the blood, although the loss of heat may go on.

*Diseases*—the most important of which are Bright's disease and diabetes—lead to a general lowering of the level of immunity, and a consequent predisposition to other diseases. We have no knowledge of the way in which they act.

There are many causes which act locally, and cause a local lowering of the resistance. Some of these have been hinted at above, but their consideration will be deferred for the present.

In considering the nature, severity, and prognosis of any disease, two factors have to be recognized: (1) the immunity of the patient,

and (2) the virulence of the infecting bacterium. A third—the number of bacteria which gain access—is also of importance, especially under experimental conditions, for it is found that, within limits, lack of virulence can be compensated for by an increase in the dose given. It is, however, one which we can rarely estimate in natural disease; besides which the growth of bacteria is so rapid that, if not checked by the resisting power of the body, a single organism would multiply in a very few hours to an enormous extent, and render it a matter of but little importance whether one or a hundred bacteria had gained access at first. The number of bacteria is probably of more importance in connection with the occurrence or non-occurrence of infection, rather of the severity of the disease when once infection has occurred. Thus we find in epidemics of typhoid fever due to water or milk that the disease is most prevalent in those who take a large amount of the infective material, but it is not necessarily more severe in them than in the patients who have apparently become infected with a small dose. This is, however, not the case with artificial infection of animals, for there the severity of the disease (in animals as similar as possible in age, weight, etc.) is fairly proportional to the dose given. But the conditions are somewhat different in the two cases, and in the artificial injection of animals we eliminate altogether the steps by which, *e.g.*, the typhoid bacillus passes the natural barriers, and gains access to the tissues.

The question of virulence is of much greater importance, and is one which must be more fully discussed subsequently, after we have seen the methods in which the host immunizes itself against the bacterium. Some general points must be mentioned here.

Cultures of the same organism, identical in all respects in morphological, cultural, and chemical characters, may differ enormously in this respect: thus a culture of streptococci may be entirely devoid of virulence to rabbits, or may be so potent that a minimal dose, containing probably but a single coccus or short chain, may be inevitably fatal. Similar facts hold for pneumococci. According to Eyre, a virulent culture may kill when 20 to 200 cocci are injected, whereas an avirulent one may fail to do so in massive doses. In most organisms there is, perhaps, not such a marked difference, but all pathogenic bacteria vary greatly in this respect, and cultures from different sources show marked variations in pathogenicity.

Further, the same culture can be made to undergo variation, its virulence being either exalted or diminished, and this is a subject of the utmost importance. An *increase in virulence* is the more difficult to secure, and can practically only be procured by passage through animals, or by other closely allied process.

Passage is carried out thus: the avirulent culture is made to infect animals either by the administration of massive doses, or by the simultaneous injection of some substance which lowers the local or general resistance (lactic acid, alcohol, the toxins of *B. prodigiosus*, etc.). In any case, the organism is made to cause an infection which may or may not be allowed to progress to a fatal issue. From the animal thus infected a second culture is made, and the material used to inoculate a second animal, and the organism will be found to have undergone a noticeable access of virulence. The process is repeated as often as is necessary, and ultimately the virulence of the culture will be brought to its highest possible pitch. The simplest method, where available, is to give the injections into the peritoneum, and to make the cultures by withdrawing some of the peritoneal fluid in a sterile pipette, and incubating it as it is, or after the addition of broth.

This method was introduced by Pasteur, and is of especial value in preparing the vaccine used against rabies. The organism of this disease is unknown, but the virus occurs in the brain, and emulsions of this substance are used for inoculation. It is found that the virus occurring naturally in rabid dogs (the "virus of the streets") is comparatively avirulent to rabbits. This is shown by the long incubation period—fifteen to eighteen days after intracerebral injection. After about fifty passages through rabbits, the virus becomes so exalted that the incubation period is shortened to six days, and the process cannot be carried further. This virus is called the "fixed virus," and its potency is maintained unaltered, no matter how many more passages are made.

Passage does not necessarily raise the virulence of the culture to all animals; it may do so only for the species used for the process, the action on other species remaining unaltered or even falling. Nor is passage necessarily followed by an increased degree of virulence—the virus of rabies diminishes in this respect when passed through apes.

Phenomena suggesting a process akin to passage occur under natural conditions. Pneumococci are frequently found in the mouths of healthy persons, and are, as a rule, of feeble virulence,

whilst those which are isolated from the lungs in pneumonia, or from pneumococcic lesions in general, are usually far more virulent. Other explanations are possible, but it seems likely that the sequence of events is as follows: The avirulent pneumococci gain access to the body owing to a temporary loss of immunity, due to one or other of the causes enumerated above, and then these are transmitted to a process in all respects like passage, the result being that they undergo a gradual increase in virulence. The struggle of the conservative forces will then be increasingly difficult, and the patient may succumb to an infection with an organism which was at first but slightly virulent. This adaptation of an organism to its environment during the course of a disease may probably be found in the future to be of great importance, as indicating a necessity for successive changes in the vaccine or serum used in the treatment of a chronic infection.

An example worthy of notice has recently been given by Ehrlich. It is not exactly on the same lines, since it deals with an alteration in the body of the power possessed by the parasite of resisting chemical agents of relatively simple composition, rather than in the power of resisting the natural forces of the body, an increase in which constitutes an increase in virulence. Ehrlich investigated the preventive and curative action of atoxyl and of various aniline dye-stuffs, such as fuchsin and trypanroth, on mice infected with trypanosomiasis. He found in a certain number of cases a cure might be obtained—*e.g.*, by feeding infected mice with fuchsin or by the injection of atoxyl—and that when this occurred the trypanosomes were not entirely destroyed, but remained latent in the body. This is a phenomenon of fairly frequent occurrence, and is called by Ehrlich, “*immunitas non sterilisans*.” After a time a relapse occurred, and was cured by a fresh dose of the drug, but after several of these recurrences this beneficial effect ceased. It was then found that the trypanosomes had been immunized or acclimatized to the agent in question—say, to fuchsin—and possessed the power of infecting mice previously treated with fuchsin and immune to ordinary trypanosomes; but the organism had not altered in its susceptibility to other dye-stuffs or to atoxyl, and mice infected with it could be cured by these agents, and not by fuchsin. Further, it was found possible to create a race of trypanosomes resistant to two or more of these agents, and these acquired characters were made permanent after several passages. If we substitute for the drugs used by Ehrlich the substances



which are developed in the body as defensive agents during an attack of disease, and imagine the same process to go on, we shall have an exact reproduction of the rise in virulence occurring during an attack of disease. If we defended ourselves against trypanosomes by the development of fuchsin, Ehrlich's fuchsin-resistant race would be extremely virulent for us.

The development of epidemics of diseases is probably due in some cases to a spontaneous rise in virulence of the infecting agent, but we have no knowledge of the causes by which this is produced.

The second method of increasing the virulence of a culture is less general, and of greater theoretical than practical interest. It consists in the cultivation of the organism for several generations in the blood-serum of an animal which has been immuned to the bacterium in question. It was discovered by Walker in the case of *B. typhosus*, and is found in the case of some other organisms. It is referred to subsequently, and we need only say here that it is allied to passage; the organism is immunized to the fluids of the resistant animal *in vitro* instead of *in vivo*. And the virulence of a culture is in general best sustained by a close approximation to the conditions of the body. Thus it is more rapidly lost at the temperature of the room than at that of the body, and the most suitable culture medium is usually one containing body fluids unaltered by heat. Thus Marmorek cultivates his virulent streptococci in broth to which one-third of its volume of ascitic fluid has been added. In the case of diphtheria bacilli the virulence (as estimated by its power of forming toxin) is best maintained by daily transplantations into broth previously raised to the body temperature, and when treated in this way shows little or no change for years.

*Diminution in virulence* occurs, as a rule, when the organism is submitted to conditions quite unlike those of the animal body, and is usually the more rapid the greater the divergence. At the same time, the growth under these conditions gradually becomes (in most cases) more abundant. The organism gradually adapts itself to a saprophytic habitat, losing in so doing its distinctive chemical properties which made it virulent as a parasite. Old laboratory cultures of bacteria which have been grown on artificial media for many generations are usually almost devoid of virulence, though here there are great variations, some species becoming inert far quicker than others.

The subject is important, since cultures of diminished ("miti-

gated") virulence are frequently employed as vaccines in the production of artificial immunity. The following are some of the chief methods employed:

1. By prolonged culture in artificial media, as described above. This method was introduced by Pasteur in the case of fowl cholera. The loss of virulence is a progressive one, and cultures ten months old are devoid of virulence.

2. By cultivating the organism at a temperature above the optimum for saprophytic growth. This was also introduced by Pasteur, and is used in preparing the vaccines to anthrax. The organism is cultivated at a temperature of  $42.5^{\circ}$  C., and all virulence is destroyed in about six weeks, though the cultures retain their power of growth unaltered. The *first vaccine* is prepared by allowing growth to continue at this temperature for twenty-four days. In appearance the bacilli are unaltered, but they have lost the power of killing rabbits and guinea-pigs, though they are still fatal to mice. The *second vaccine* is cultivated at a high temperature for a fortnight only; it is virulent to mice and guinea-pigs, but not to rabbits.

The process may also be carried out by a short exposure to a higher temperature. Chauveau's vaccine consists of blood containing anthrax bacilli, heated to a temperature of  $50^{\circ}$  to  $55^{\circ}$  C. for ten or fifteen minutes. The bacilli remain alive, but are mitigated in virulence.

3. In some cases the addition of various chemical antiseptics in minute amounts to the culture medium has a similar effect. This is the case with anthrax also. Addition of chemical substances is also made with the idea of destroying toxins, but this is a different phenomenon.

4. The virulence may be destroyed by *drying*. This method was introduced by Pasteur for the preparation of a vaccine against rabies. We have already described the method by which he obtained the fixed virus and its action on rabbits. He found that by suspending the spinal cords of rabbits dead of this fixed virus over caustic potash at a temperature of  $23^{\circ}$  C. the virulence was entirely removed in fifteen days. Drying for a shorter time diminished the virulence, but did not remove it entirely.<sup>1</sup>

<sup>1</sup> The more modern idea is that the process of drying kills off a large number of the pathogenic organisms, and that the use of the dried cord is merely another method of giving very minute doses of virus of normal strength.

5. In some cases (as has been noted above) passage through animals diminishes the virulence. In some cases this can be exalted by passage through a series of animals of one species and diminished by the use of another. Pasteur showed this to be the case in swine erysipelas, the potency of which (as tested on pigs) is increased by passage through pigeons and decreased by passage through rabbits. Cultures thus attenuated are used as vaccines.

The term ACQUIRED IMMUNITY is one that is used to denote an increased resistance to an organism dependent on some modification in the animal's constitution due to some definite process to which it is subjected, but not including the modifications due to improvements in the general health due to betterment of the environment. For example, a person living in insanitary surroundings will undoubtedly acquire a higher degree of resistance to the tubercle bacillus on being moved to more healthy ones, but we do not speak of that as acquired immunity. The distinction is this: The elevation of the natural resisting powers due to improvement in the general vitality is a more or less general one, and affects the immunity to most or all bacteria almost equally; whereas in acquired immunity in the narrower sense, to which the use of the term is restricted by pathologists, the alteration is in the powers of resistance to one bacterium only. For example, a debilitated person removed to a more healthy environment, given better food, tonics, etc., would become more resistant to the attacks of smallpox, and to other diseases as well; we should speak of that as an augmentation of the natural immunity. But after an attack of smallpox, or after vaccination, his immunity to smallpox is enormously increased, whereas his resistance to other organisms is unaltered; this is acquired immunity.

This is expressed by the use of the word *specific*, embodying an idea difficult to define, but implying a direct relationship of cause and effect, and, moreover, that a certain effect is only produced by a certain definite cause. Thus the toxin of diphtheria is specific for the diphtheria bacillus in the sense that it is produced by that organism, and by no other; diphtheria antitoxin is specific for diphtheria toxin, since it is produced only as a result of the injection of that substance; the reaction caused by the injection of tuberculin into a tuberculous animal is specific, etc.

In most instances there is another difference between a rise in natural immunity and the development of acquired immunity, in that the latter is much stronger. Thus, the power of resistance

to smallpox of a perfectly healthy person is probably not great, whereas that produced by an attack of the disease or by vaccination is for a time almost absolute. Yet all degrees of acquired immunity exist, from the very slight amount which is developed during an attack of pneumonia, and which is probably only just sufficient to cut short the disease, to the enormous degree that can be obtained in animals hyperimmunized to diphtheria or tetanus toxin or hypervaccinated to *B. typhosus*. Perhaps our conception of immunity in the past has been influenced too strongly by a study of these latter conditions, which are readily induced in the laboratory, but rarely if ever seen in the actual practice of medicine. They represent in an extreme form the changes which follow disease of natural origin, and possess the theoretical interest which attaches to all extreme cases.

ACQUIRED IMMUNITY occurs in two distinct forms—*active* and *passive*. A third form exists, which we may call *mixed*, since it is brought about by a combination of the procedures necessary for the development of the other two.

Active immunity may be defined as acquired immunity, due to an attack of the disease in question in its normal form, or in a modified and less severe form of artificial production. The essential feature is that the cells and tissues of the person or animal should be subjected to the action of the bacterium (or its toxin), and by its own efforts, and as a result of an active struggle with it, should become less susceptible to its toxin than before. Active immunity is developed only as a result of an illness of the host, due to the action of the microbe on its cells; and this illness may be of any degree of severity, ranging from an unmodified attack of the disease which may threaten life down to the most transitory and unimportant reaction due to an injection of a minute dose of a mild vaccine. And one of the great aims of modern preventive medicine is to reduce the severity of the disease necessary to produce acquired immunity to a minimum. The greatest step ever made in this direction was Jenner's substitution of vaccination for inoculation. In each case the effect is the same as regards the resulting immunity (though in different degree), but the disease in the former case is mild and devoid of danger, in the latter severe and dangerous. As a general rule, it may be taken that the severer the disease the stronger and more lasting the acquired immunity. A good example will be quoted when dealing with mixed immunity. This is not necessarily the case,

however, for the repeated injections of vaccines which are so mild as not to cause any noticeable general and very little local reaction may induce a high degree of immunity.

The main methods in which active immunity is acquired are these :

1. A natural attack of the disease, or an attack which is natural in course, but of artificial induction. The only example of the latter in human medicine is the now disused practice of smallpox inoculation, in which the person to be protected was inoculated with the disease, which ran a perfectly usual course, and was not infrequently fatal. As a rule, however, it was milder than the naturally acquired smallpox, since the infective material was taken from a favourable case, and the operation performed when the patient was in good health and able to get proper attention from the outset. Probably, too, the severity of the disease was somewhat modified by the fact that the virus did not reach the body by the usual route. But the infection was ordinary smallpox, and might start an ordinary epidemic.

The process is used to a much greater extent in veterinary practice, where an occasional death due to the induced disease is of comparatively little importance if thereby the outbreak can be controlled or the great majority of the flock saved. As a rule, an attempt is made to render the attack as mild as possible, either by (a) limiting the amount of the infective material used, or (b) by introducing it in an abnormal way, or (c) inoculating animals at a time when they are found to be least susceptible, or by a combination of these methods. Thus Texas fever is a disease of cattle due to a protozoon (*Piroplasma bigeminum*) which is conveyed by the bites of ticks. One of the methods used for the protection of cattle in infected districts is to expose calves whilst still milk-fed to the bites of a few infected ticks; another is to inject blood from diseased animals (containing the parasite) in small doses direct into the jugular vein. In favourable cases the result is a severe attack of the disease, which, however, is rarely fatal, and is followed after a time by complete immunity. In some cases the disease is but slight, and in them a second or even third dose, in each case larger than the preceding, is required. The mortality from the injections is from 3 to 10 per cent., whilst that of untreated animals in infected areas is about 90 per cent.

A similar method is in use for combating rinderpest, but here bile from an animal dead of the disease is used as the infecting

agent, since the blood frequently contains other infective materials which would complicate the issue.

In pleuro-pneumonia of cattle the severity of the disease is lowered by altering the route of infection. In the natural disease the infection probably enters by the lung, and its course is severe and dangerous. Protection is conferred by inoculating virus from the lung of an animal dead of the disease into the subcutaneous tissue near the tail; much local swelling results, and general immunity is established. Perhaps, strictly speaking, this method of induction of active immunity should be put in a class of its own; it is one in which a local is substituted for a general disease, with the obvious result of greatly lessening its severity.

The material used in the production of artificial immunity of the type we are describing is sometimes called a vaccine. This is undesirable, and it is advisable to use the word *virus* for material containing the infective agent in its normal virulence, retaining the word *vaccine* for that in which the bacterium has entirely lost its power of producing the normal disease, whatever the dose and whatever the channel of introduction. The term is a somewhat unfortunate one etymologically, but it is in such general use that it is hopeless to attempt to displace it.

2. By the use of living cultures of pathogenic bacteria of diminished or altered virulence—*i.e.*, of a living vaccine. There are as many modifications of this method as there are ways of mitigating the virulence of a culture, and different methods are applicable to different diseases.

(a) By the use of vaccines diminished in virulence by passage through animals. The most important example of this is, of course, Jennerian vaccination. It would take us too far to examine the evidence in favour of this view, but it may be taken as fairly proved that ordinary lymph vaccine consists of a culture of the smallpox organism modified by passage through calves, the modification being of such a nature that it has lost its power of producing a general disease (smallpox), but retained that of causing a local one (vaccinia) otherwise similar in nature.

We have already referred to the decrease in the virulence of the bacillus of swine erysipelas on passage through rabbits, and the use of these mitigated cultures as a vaccine for pigs. This is a better example of the type of immunity we are considering, since it has to do with a known organism.

(b) By the use of vaccines in which the virulence is diminished

by drying. The only practical example is rabies. There the process of immunization is carried out by means of the use of a series of vaccines of gradually increasing degrees of virulence, the degree dependent on the time for which drying has gone on. It is, of course, necessary to proceed with extreme caution, since the cords that have been dried for but a few days are still infective and virulent, and the amount of natural immunity in man is extremely small, so that an attempt to accelerate the process might be fatal. The method varies somewhat at different laboratories, but the following may be taken as a type of the procedure used. It is of interest as being the method used in the first case treated—that of Joseph Meister.

*Day 1.*—Inoculation with vaccine made by drying the cord for fourteen days. A second injection with cord treated for ten days.

*Day 2.*—Two injections; cords dried for eleven and nine days.

*Day 3.*—One injection; cord dried for eight days.

*Day 4.*—One injection; cord dried for seven days.

*Day 5.*—One injection; cord dried for six days.

*Day 6.*—One injection; cord dried for five days.

*Day 7.*—One injection; cord dried for four days.

*Day 8.*—One injection; cord dried for three days.

*Day 9.*—One injection; cord dried for two days.

*Day 10.*—One injection; cord from a rabbit which had died the same day, and which was therefore unaltered in virulence.

The method in use in France at the present day is almost like this, except that the latter stages are repeated twice, or, in severe cases, three times—*i.e.*, on the ninth and fourteenth days in mild cases (and on the nineteenth also in severe ones) injections of nine-day cords are started, and the strength increased rapidly, so that three-day cords are used on the thirteenth, eighteenth, and twenty-first. In Germany the treatment is begun with eight-day cords, the older ones being considered inert.

(c) By the injection of living cultures modified by heat. The classical example is vaccination against anthrax by means of Pasteur's two vaccines, the method of preparing which is given on p. 18. The first vaccine is injected, and is followed by the second in about a fortnight, immunity being established in about another fortnight.

(d) By the injection of cultures attenuated by prolonged cultivation *in vitro*. The use of this method in the case of chicken

cholera has been referred to already, and it is the one usually employed in the laboratory, where old cultures are used in preference to more virulent ones in the early stages of immunization.

(*e*) By the use of very small doses of living cultures of full virulence. This has been proved possible in anthrax, symptomatic anthrax, and some other diseases. At present the process is more interesting than practically useful, but it has been used clinically in the case of tubercle, treatment being commenced by the injection of a single living bacillus, and promising results have been obtained.

3: A third class of methods consists in the use of vaccines composed of dead bacteria. The advantages are obvious: the dose is under accurate control; the disease which it induces is self-limited, so that it is impossible for a general infective process to be produced when used on a person of deficient natural immunity; and the vaccine is easy to keep in a condition ready for immediate use. Hence this method is mostly used in human medicine, whereas the use of mitigated or unmitigated viruses is mainly confined to veterinary work. The methods used in the preparation of the vaccines varies greatly in the different cases, and here we can only glance at some of the general principles. In preparing the cultures, the most careful precautions have to be taken to insure the purity of the microbe used and absence of all other pathogenic forms, especially perhaps the spores of the tetanus bacillus. The age of the culture has to be determined by the necessities of the case, but as a rule young cultures are preferable. The method by which the bacteria is killed also varies, but heat is generally employed, and as a rule the shorter the exposure and the lower the temperature the better. In other cases the bacteria are emulsified in saline solution and allowed to undergo autolysis at the body temperature, sterility being ensured subsequently by means of heat or chemical antiseptics; or they may be killed with a minimum of heat, and submitted to autolysis at 37° C. subsequently.

There are numerous methods of determining the dose to be used. (*a*) A definite fraction of an agar or other culture of known age may be taken, or, what comes to much the same thing, the growth from so many square centimetres or millimetres of surface of the culture medium. (*b*) The amount may be judged by the weight, and this is the method used in the case of tubercle. When it is employed with other bacteria it is usually



carried out by means of standard loops, each of which will pick up a known amount of growth. (c) In Wright's ingenious method of counting a vaccine a certain amount of the latter is mixed with human blood in definite proportions, and films are prepared and stained. The numbers of red corpuscles and of bacteria in several fields of the microscope are then counted, and (the numbers of red corpuscles in a definite volume of the blood being known) the proportions of the two will permit of the calculation of the numbers of the bacteria. (d) Some determine the strength of the vaccine by reference to a permanent standard, usually consisting of a fine suspension of barium sulphate. A strong emulsion of bacteria is prepared and diluted until it matches the standard. (e) The volume of the bacteria in the emulsion may be determined by centrifugalization in a graduated tube, and a certain volume of sediment made up to a certain volume of vaccine. (f) The number of bacteria present in the emulsion may be counted directly by the use of the counting chamber of the hæmocytometer, and this is the method I usually employ. The emulsion is diluted (usually to twenty times its volume) with a dilute solution of methylene blue or other stain, boiled, and a drop placed in the counting chamber and prepared as if it were a blood specimen in which the red corpuscles were to be counted. A  $\frac{1}{8}$ -inch lens and a high eyepiece are used, and, as a rule, the process presents no difficulty.

In all cases an addition of a chemical antiseptic is advisable to avoid subsequent contamination. Carbolic acid or lysol (0.25 to 0.5 per cent.) are most used; another good plan is to keep a few drops of chloroform at the bottom of the bottle, so that the fluid is always saturated.

This method is mostly used in plague, cholera, and enteric fever in preventive medicine, and in the treatment of infective processes by Sir Almroth Wright's method in curative medicine. These will be discussed subsequently.

4. Inoculation with the chemical products or with the toxins of the bacteria, the bodies of the bacteria themselves being removed by filtration or in some other way. This is obviously closely allied to the last method—the use of killed cultures.

It was introduced by Smith and Salmon in the case of hog cholera, and is now chiefly used in the immunization of animals for the production of antitoxic sera. It is considered fully in a subsequent chapter.

PASSIVE IMMUNITY, the second form of acquired immunity, is conferred by injecting into a susceptible animal the serum of one which has acquired an active immunity to the disease in question. It is a kind of second-hand immunity, acquired in virtue of the reception of substances actively formed by another animal which has had to fight against the infecting agent in order to form them. In its production there is no necessary illness, however slight. Such may occur, it is true, but it is not more than would be produced by normal serum, and stands in no necessary relationship to the development of the immunity. And when such illness does occur, it does so after the production of the immunity, and may be very severe when the protection given is but slight, and *vice versa*.

For the production of passive immunity it is necessary to inject the serum of an animal which has been artificially immunized, that from one which is naturally immune being devoid of action in this respect. To this general rule there are one or two exceptions, which are perhaps more apparent than real.

Passive immunity is sometimes called antitoxic. The term, however, is not a good one, since there are several varieties of passive immunity, only one of which is due to an antitoxin.

Passive immunity is specific—that is, the serum of an animal which has acquired immunity against one organism will protect a second against that, and against no other. In this, of course, it resembles active immunity, but the two differ in several important particulars.

1. As regards its production. Active immunity takes some time—usually a week or so—to develop, dating from the infection or injection of the vaccine, and in many cases at least its appearance is preceded by a negative phase, in which the natural immunity to the organism in question is lowered. But passive immunity is established as soon as the serum has become mixed with the blood of the person or animal injected, and there is no negative phase.

Hence in severe infections our best hope in the way of specific medication is in the production of passive immunity. It is but recently that the injection of vaccines was thought of in face of an infection already developed, and it is obvious that the method will be useless or dangerous in very severe and rapid infective processes. Passive immunity, on the other hand, can be induced

at once and without a negative phase. Unfortunately, it is not always or often possible.

2. As regards its duration: Active immunity lasts for a long time, the length differing greatly in different diseases and after various methods of induction. In many cases it lasts a year or more. Passive immunity, on the other hand, is always of brief duration, and lasts only about as long as the serum injected is



FIG. 2.—SHOWING THE SEQUENCE OF EVENTS IN THE PRODUCTION OF ACTIVE IMMUNITY.

An injection of vaccine at *b* is followed by a decrease in the degree of immunity (negative phase), a rise, and a gradual return to the normal condition.

actually present in the blood. It depends to a certain extent on the dose of serum given, and also on the species of animal from which it was derived. An animal of a certain species is immunized for a longer period by serum from another animal of the same kind than from one of a different species. In general terms the duration of passive immunity is three to six weeks. It is renewable at pleasure, as far as we know indefinitely.

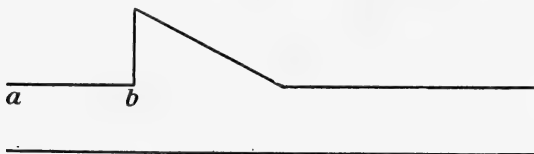


FIG. 3.—SHOWING THE SEQUENCE OF EVENTS IN PASSIVE IMMUNITY.

An injection of serum is given at *b*.

Hence passive immunity is chiefly of value to ward off an infection the danger to which is of short duration. Thus in veterinary practice the passive immunity of horses conferred by the injection of tetanus antitoxin is of the greatest possible value before operations, or immediately after the infliction of a wound, horses being so prone to tetanus that in some places any opera-

tion was a matter of great danger before the introduction of this method.

Passive immunity is also useful as a basis for active immunity. This will be described under the heading of Mixed Immunity.

3. Passive immunity for a given bacterium or its products cannot be made so potent as the active form for the same disease in the same animal species. The reason is obvious: the passive form only occurs in virtue of the presence in the blood of some of the foreign serum, which can never form more than a fraction of the whole fluid. The degree of the immunity may be sufficient for all practical purposes, but can never reach the enormous height met with in hypervaccinated animals.

4. Active immunity we believe to be developed to some extent in all, or almost all, infections, but the production of passive immunity is impossible in very many cases—*e.g.*, tubercle (as far as we know at present), infections with pyocyaneus, glanders, malaria, and many other parasitic organisms. Perhaps in the future we shall be able to procure active sera against all organisms, but at present we have comparatively few of any value.

MIXED IMMUNITY is a combination of the two forms already described, in which the dangers and delay incidental to the induction of active immunity are avoided by the use of a protective serum. It is really a succession of the two forms, the passive immunity being developed at once as a consequence of the injection of the serum, whilst the active form develops later in consequence of the vaccination. The process is sometimes called sero-vaccination.

It is not of great importance in human pathology, the chief example being the form of typhoid inoculation suggested by Besredka, and not yet used on a large scale. In it the killed typhoid bacilli are submitted to the action of the immune serum, from which they absorb certain protective substances and become modified thereby. It is claimed that this treatment prevents the development of the discomfort that follows the use of ordinary typhoid vaccine, and that the immunity is developed very rapidly. It may be followed by an injection of ordinary vaccine.

The method is used to a considerable extent by veterinary surgeons, and there are several modifications in the process, the serum being injected either mixed with the virus, or before, or after, or simultaneously in different sides of the body. Thus in the treatment of South African horse-sickness the virus (the blood

of diseased animals) may be mixed with the serum from hyper-immunized animals and injected subcutaneously. If the serum and virus are injected separately the animal will in all cases acquire passive immunity; but unless there is some degree of illness (a "reaction") this will be but temporary, and no active immunity will be superadded. Thus, if the serum be injected and the virus given subcutaneously at the same time, no reaction follows, and the immunity does not last more than a month; but if the injection is made into a vein a reaction occurs, and active immunity, lasting for about a year, will follow (Stockman).

The method is also used in the early stages of antitoxin formation, the horse being treated with a mixture of toxin and antitoxin, the latter being in excess. But here it seems unquestionable that active immunity is acquired, and the mechanism by which this occurs is discussed subsequently.



FIG. 4.—MIXED IMMUNITY.

The presence of a negative phase, as shown in the diagram, is not essential.

LOCAL IMMUNITY.—We have hitherto spoken of the body as a whole, assuming that all parts are equally resistant or susceptible. This is not the case, and certain parts are found to have a marked degree of immunity to certain bacteria. Here we have to be sure that we are dealing with regions that are equally exposed to infection. The stomach, for example, is comparatively rarely attacked by infective processes, and this may be due to the fact that the gastric juice is of a sufficient degree of acidity to kill or inhibit most bacteria. Yet here it is probable that this does not account for all the phenomena, and that some degree of true local immunity does exist. Numerous other examples may be quoted. Pneumococcic infections are common in the lungs and pleura, but rarely spread further, and cause disease of the ribs and intercostal muscles; tubercle is common in the bones and extremely rare in the muscles, whilst *Trichina spiralis* affects the muscles and never

attacks the bones, and rarely any other tissues. Some of the best examples may be taken from diseases that spread by continuity from one tissue to another. Thus the gonococcus in either sex spreads along the urethra with ease, but seldom involves the mucous membrane of the bladder; it practically never attacks the vaginal mucosa (in adults), but spreads from the cervical to the corporeal endometrium, and thence to the Fallopian tubes, but comparatively rarely goes farther and produces a general peritonitis. Diphtheria, too, though it may spread in any direction, seldom creeps down the œsophagus. Many other examples might be quoted.

There are marked differences in regard to local immunity between the child and the adult. The most marked example, perhaps, is in the almost perfect local immunity of the scalp to ringworm in adults, which contrasts so markedly with the absolute susceptibility of children, whereas the susceptibility of the skin of the body to the same parasite is, if anything, greater in the former. In most cases of differing immunity at different ages the child is more susceptible, just as its resistance to general diseases is less, and the few exceptions that may be quoted are perhaps rather apparent than real.

Local immunity may be natural or acquired. Passive immunity, of course, cannot be local for long, as any serum which is injected will rapidly diffuse away and be removed by the lymphatics and blood-stream. The cases mentioned above are all examples of natural local immunity. The difference between the reactions of the tissues of children and adults do not necessarily point to the acquisition of any active immunity in the sense in which the word has been defined above, but rather to the general rise in resisting power accompanying the general improvement in strength and vitality, and in some cases, perhaps, to an actual maturation of the tissues, as in the case of the adult vaginal mucous membrane, which is immune to the gonococcus, whereas the thin and immature infantile membrane is susceptible. The immunity of the adult scalp to ringworm also is not acquired, using the word in the narrow sense, for it occurs apart altogether from an attack of the disease.

Our knowledge of acquired local immunity is very incomplete; it is a difficult subject for research, and more attention has been paid to general immunity. A little consideration will demonstrate the fact of its occurrence. For example, when a person develops

crops of boils it will often be found that one is undergoing involution whilst another is developing; hence the cure of the first cannot be due to any general immunity, but must depend on local changes which do not affect the second. A similar line of argument will show the development of acquired immunity to the streptococcus in erysipelas; the healthy skin is susceptible, since the disease spreads to it, but the process does not extend backward into an area already affected, but now cured, or does so but rarely.

The subject cannot be discussed further with advantage, and will be deferred to a subsequent chapter, when the known factors on which immunity depends have been elucidated.

There are important non-specific causes for alterations in local immunity, as is the case with general. These practically resolve themselves into the presence or absence of an adequate supply of blood; the more copious the supply of healthy circulating blood, the greater the resistance to infections, and *vice versa*. Hence the utility of fomentations and other hot applications in the initial stages of an infective lesion; hence, too, the application of Bier's method of passive congestion, in which an excess of blood (though partly stagnant) is made to flush the tissues. And there is no doubt that the object of the dilatation of the vessels and acceleration of the flow of blood through them which occurs in the early stages of inflammation is a beneficial process which has this improvement of the local resisting powers as one of its objects, the influx of an increased number of leucocytes and the dilution and removal of the soluble toxins being others. In acute inflammation we may distinguish two stages. In the first, the stage just mentioned, the conservative reaction of the vessels is most obvious, and in the case of a mild infection, or if the immunity is very strong, may suffice to destroy and remove the infective material and its toxin. The stagnation and ultimate cessation of the blood-flow are indications that the irritant is, temporarily at least, getting the upper hand, and, by cutting off the blood-supply, is neutralizing the most powerful defensive factor. The acceleration of the flow may be regarded as physiological, the retardation and cessation as pathological.

The causes of local reduction of immunity by obstruction of the blood-stream are numerous, the most important being traumatism (by injuring the vessels going to the region), endarteritis, thrombosis, tight bandaging, etc. They need not be discussed at

length, but it is advisable to point out that severe traumatism, in the form of violent laceration and contusion of a part, is an extremely powerful predisposing agent, and that it acts in two ways, or perhaps more. In the first place, there may be some death of tissues, either in small or large amounts, and in these dead tissues the natural resisting powers are of course in abeyance, so that the bacteria will grow unchecked, as they would in dead culture media; and, secondly, that the blood does not reach this dead material, and the leucocytes only do so with difficulty. The importance of this is well seen in tetanus. The normal tissues have a considerable degree of resistance to this organism, and infection rarely takes place in a clean incised wound, even in cases in which we can be almost certain that the spores of the tetanus bacillus have been introduced.

Another cause of reduced local immunity is the action of irritants on the tissues. Here we must distinguish two cases. If the irritant be but mild, it may be actually beneficial; it causes the earlier phenomena of inflammation which we have previously referred to as being protective, and may tend to raise the resistance of the part in consequence. Thus, according to many observers (who do not agree precisely on the interpretation), the injection of a small quantity of almost any bland (but nevertheless foreign) substance into the peritoneal cavity may protect an animal against a lethal dose of a bacterial culture introduced subsequently; normal saline solution, water, broth, serum, etc., all have this action. But if the irritant be more powerful, so that the tissues are killed and the vessels occluded, or the leucocytes killed, the susceptibility of the region is greatly increased. Chemical antiseptics have this action, especially in certain regions, such as the peritoneum. The same thing may be demonstrated experimentally. Tetanus spores washed free of toxin will not produce tetanus in rabbits, but will do so if an irritant such as lactic or carbolic acid is injected simultaneously.

A few words may be said here on the phenomena of immunity and susceptibility in relation to the modifications they cause in the infective processes. Where the immunity is great, or, as we say, absolute, the result of an injection of the infective agent is nil; there is, of course, some degree of inflammation, but this follows the injection of any fluid, even normal saline solution, and the effect of the bacteria themselves is inappreciable. In this case, therefore, the bacteria are immediately destroyed, and the



substances which they produce are without deleterious effect on the cells of the body. In another group of cases, referred to above, the bacteria do not die, but their toxins remain harmless to the host; this is Ehrlich's *immunitas non sterilisans*, and it occurs in the case of many of the lower animals which have in their blood various protozoa (trypanosomes, etc.), without thereby suffering the slightest appreciable injury. In man the condition is best seen in its acquired form in the immunity possessed by negroes to the action of malaria parasites, though the plasmodium may be found in the blood. A closely allied phenomenon is in the *latency* of bacteria. Thus a person may develop an attack of typhoid osteitis years after an attack of typhoid fever, and we can only assume that the bacteria have lain latent in his tissues for this time; in all probability they have been kept from infecting him as a result of a sufficient degree of immunity, and when this breaks down or wears off a renewed outburst occurs. The gonococcus may be latent in a similar way for periods equally long. Another similar phenomenon is the carriage of infection by persons who remain themselves healthy. Diphtheria is a common example, and it is no rarity to find a person in whose throat diphtheria bacilli are present, but who remains unattacked. Here the immunity suffices to prevent the bacillus from invading the body, but not to destroy it.

At the opposite end of the scale occur those cases in which immunity is practically absent. Here the result of the introduction of the bacteria is a rapid infection, both local and general, with profound symptoms of intoxication; the bacteria spread through the tissues just as they would through a good culture medium, and, in addition, invade the blood and multiply therein. This is rarely seen in man, though some examples of septicæmic plague and streptococcal septicæmia from post-mortem wounds approach it closely. It can be produced experimentally in animals, when large doses of virulent cultures are injected. Death follows in a few hours, and the blood is found to be swarming with bacteria.

Between these two extremes come those cases in which the introduction of the bacterium is followed by the production of a local lesion. This *always* indicates some degree of local immunity, and may be regarded as an attempt to localize the organism and prevent its further spread. And the nature and severity of the local lesion stand in close relation to the severity of the infection

and the degree of the immunity. For example, in severe and rapidly fatal infections from post-mortem wounds—*i.e.*, where the infection is virulent and the immunity but slight—there is very little local reaction and very little glandular enlargement, the process being septicæmic from the first. Where the infective and protective forces are equally matched the local lesions are more developed; inflammation, and usually suppuration, occur at the site of the wound, and the glands enlarge and may suppurate; and when the infection is so feeble as to be quite unable to cope with the immunity, the local lesion is the sole result of the infection. Eyre gives a similar example in the results of injecting similar doses of pneumococci into rabbits of different ages. The young animal is most susceptible, and in it death occurs within forty-eight hours from septicæmia, and there is but little local reaction. In half-grown animals the local lesion is more developed, and is gelatinous or fibrinous, containing many leucocytes, and the animal lives several days. In old rabbits quite definite pus is formed, and the animal lives longer, and may recover completely. Hence suppuration may be regarded as a proof that the defensive and infecting forces are fairly balanced, and that either may be victorious in the conflict.

The other local lesions need not be discussed at length, but the case of tubercle and the allied diseases requires a brief notice. Here the lesion indicates the presence of a very considerable degree of immunity to the toxin, for the structure of a tubercle is exactly similar to that of the cellular reaction to many feebly irritating foreign bodies—*e.g.*, unabsorbable ligatures, substances from which it is clear no potent toxin can be given off; but it also indicates that there is a defect in the mechanism by which the bacilli should be removed, since the process is (for a time at least) a progressive one. Here the walling-in of the infected area which occurs as the result of the reaction of the tissues may be taken to be a defensive process, but, as we shall have occasion to see, it is one of doubtful utility.

EARLY THEORIES OF IMMUNITY.—Before turning to the discussion of the nature of immunity in the light of our present knowledge, it will be convenient to insert a short account of some of the early theories of the subject, which are in the main of historic interest only. They have served their purpose as a point of departure for subsequent research.

Of such nature was Pasteur's theory of exhaustion, the earliest

attempt at a scientific explanation of the facts of recovery from, and subsequent immunity to, the infectious diseases. Pasteur was a chemist, and was only led to the study of bacteriology by the pursuit of chemical investigations into examining reactions which he proved to be due to micro-organisms. His theory was a chemical one. A certain amount of food is necessary for each bacterium, and when the total amount contained in a given solution is used up the growth of the bacteria must cease. For example, if we take a *dilute* solution of sugar (containing the necessary salts, etc.), and inoculate it with yeast, the cells will begin to divide and multiply with great rapidity. After a time the growth ceases, and it will not be resumed if we inoculate the fluid with an additional amount of yeast. We may compare the test-tube to the patient, the yeast to the pathogenic organism, and the process of fermentation to the disease, and we may say that the fluid has recovered from the disease and is now immune to it. This immunity depends upon the absence of sugar, which was used up by the yeast cells, and if more sugar be added the process of fermentation may be restarted by a fresh inoculation, or by the yeast still remaining.

The theory can easily be disproved, from the fact that bacteria may grow well enough in the dead tissues and fluids of immune animals; and, secondly, because immunity, as we have seen, may be produced (in some cases) by the injection of the chemical products of the bacteria, substances which can hardly use up food materials. The theory has, however, been recently revived in a modified form by Ehrlich, who considers that there is sufficient evidence for the occurrence of this form of immunity in certain cases. He calls it *atreptic* immunity.

The retention hypothesis of Chauveau is the exact opposite of Pasteur's. Several observers showed that the growth of micro-organisms in fluid media might cease spontaneously whilst abundant food material remained unutilized. This was found to be due to the presence of certain products of metabolism, which, like carbon dioxide in the case of animals, act as poisons to the organism which produces them. For instance, the fermentation of sugar by yeast is found to cease when about 14 per cent. of alcohol is present, and if a strong solution be taken the process will stop at this point, but can be started again if the alcohol be removed by distillation. Here the fermentation is stopped by alcohol, a product of metabolism of the yeast cell, which acts as a

poison on the organism producing it. The theory of immunity based on these facts is obvious. Bacteria growing in the body will yield substances inimical to the continued growth of the organism, so that they will die out and recovery ensue, and the body will remain immune as long as these substances are retained therein. This theory accounts well for the production of immunity by injections of the toxins and other soluble products of bacteria. It is negatived by the fact that bacteria may grow in the blood and tissues of immune animals, and is improbable if we consider that immunity may last for many years, and that it is extremely improbable that substances (necessarily soluble) should be retained in the body for so long a time.

We shall now proceed to a study of the more modern views, and in doing so it will be convenient to deal with the subjects of immunity to toxins and immunity to bacteria in separate sections. Of course, in most cases they run parallel to one another: an animal contracts a disease because its fluids and tissues cannot kill the pathogenic bacteria offhand, and because its cells are susceptible to the action of the toxin, and *vice versa*. This is not necessarily the case, however, and the two phenomena may be entirely independent.

The subject of immunity of toxins is on the whole the more important of the two, the simpler (though complex enough), and the best understood. It will be best to deal with it first.

## CHAPTER II

### ON THE NATURE OF TOXINS

THE fact that the pathogenic action of any organism is dependent entirely, or almost entirely, on that of the toxins which it produces renders it necessary to make a brief study of these substances before considering the method in which the infected animal reacts to the organism, and defends itself against infection. In doing so we must distinguish clearly between the *specific toxins* which are produced by any organism and the non-specific and less important *poisons* which it may also elaborate. The difference is a fundamental one. Numerous bacteria produce by-products of metabolism, excreta, etc., which are comparatively simple chemical substances of definite composition; for example, acids, alkalis, alcohol, ptomaines, nitrites, etc. These may be poisonous, and may, in some cases at least, play a part of some importance in the production of the symptoms of the disease. The cholera vibrio, for instance, produces nitrites in considerable amount, and since the symptoms of cholera have some resemblance to those of nitrite poisoning, it is conceivable that those substances may be, to some extent at least, the active causes of the disease, and these nitrites might be regarded as the toxins of the cholera vibrio. This, however, is not the case, and the true toxins are quite different in nature, as is shown by many facts, especially by the proof that cholera vibrios which have no longer the power of producing nitrites may still cause infection in susceptible animals.

The specific bacterial toxins differ from these poisonous substances in many important particulars. They are, as a rule, formed only in very small amounts, and are extremely powerful. For example, the toxin of tetanus may readily be obtained in so poisonous a solution that  $\frac{1}{10000}$  c.c. will kill a guinea-pig in a day or two, and of this solution only a very small fraction even of the dried residue consists of toxin. They are not simple chemical

substances, and their exact nature is as yet unknown. This may be due in part to the minute amounts which are formed, and in part to the difficulties which prevent their being obtained in a pure state; but there are other reasons, to which we shall revert later, for this complexity. Further, they are, with a few exceptions, very fragile substances, and are readily destroyed by the action of many agents, and especially by heat. Nearly all the bacterial toxins are rendered inert by boiling, and many of them by a short exposure to a temperature of  $60^{\circ}$  or  $70^{\circ}$  C. They are usually destroyed by gastric digestion, so that they are without action when administered by the mouth.

A considerable amount of attention has been paid to this question, since it would be desirable, if possible, to replace hypodermic injections of vaccines, etc., by oral or rectal administration. In general terms the statement made above holds good: toxins administered by the mouth are not absorbed as such, and do not produce the characteristic symptoms of the disease. In some cases, however, there is reason to believe that a small amount of absorption, probably of the toxin in an altered form, does occur, and a certain degree of immunity may be produced by the oral administration of killed cultures of typhoid bacilli, and possibly of tubercle bacilli. But this method has only one advantage—its painlessness—over the hypodermic method, whereas its uncertainty renders it extremely undesirable. There can be no doubt that the advantage of giving an exactly measured dose, with the certainty that every particle will be absorbed and act in the way desired, will, under ordinary circumstances, render the hypodermic method infinitely preferable. To administer infinitesimal doses of killed tubercle bacilli or of TR to an infant who may be swallowing large doses of living and dead bacilli in milk, sputum, etc., does not appear rational, and the clinical evidence in its favour is entirely unconvincing. In the case of ricin, about a hundredth part of the toxin given by the mouth is absorbed as such—*i.e.*, the minimal lethal dose on oral administration is about 100 times as large as the lethal dose of the same preparation given subcutaneously (Stillmarck). Ricin is, however, far more resistant to the action of digestive enzymes than are the exotoxins.

The most important feature of the bacterial toxins is their relation to immunity. It is possible in all cases to render a susceptible animal immune to their action by the injection of the toxins in suitable doses at suitable intervals, though in some

cases the task is a difficult one. This is not the case with the non-specific toxins. It is true that in a few isolated instances we are able to increase slightly the resistance of an animal to the simple chemical poisons (*e.g.*, to alkaloids such as morphine), but these apparent exceptions hardly interfere with the utility of the general rule. Further, and more important, *an animal immunized to the action of a toxin is also protected against the pathogenic action of the bacterium which produces it, and vice versa.* Thus an animal which has been rendered immune to the toxin of tetanus by repeated injections of that substance is also immune to infection with the living cultures of the bacillus, and an animal which has successfully survived an infection with the tetanus bacillus is thereby rendered in some degree immune to the action of tetanus toxin. This method of immunization with the bacterial toxins (the so-called "chemical vaccination") is of the utmost importance in practice. It was introduced by Smith and Salmon, who showed that it was possible to immunize pigeons against living cultures of the hog-cholera bacillus by means of the sterilized products of that organism.

When this method is applicable it supplies us with a test as to the specificity of a toxic substance which we have isolated from a culture of a bacterium, or from the organs of an animal which has been killed by an infection. The substance must be poisonous for animals which are susceptible to the infection in question, and it must be harmless to animals which have been immunized to the organism; on the other hand, it must immunize animals both to its own action and to that of the bacterium when injected in a living state. These conditions are never fulfilled by the non-specific toxins.

There are a few apparent exceptions to this rule, but they fail to stand investigation, being based on the fact that it is easier to render an animal refractory to a living organism than to its toxin. Thus an animal which has been injected with the filtered products of certain organisms may be rendered immune to infection with those organisms, but remain as susceptible as before to their toxins. But this is due to the fact that the animal has been immunized but partially; if the process be carried further the animal will be rendered refractory to both.

Again, an animal which has been immunized to the toxin of one bacterium remains as susceptible as before to the action of another toxin or bacterium. A horse which has been immunized

to diphtheria toxin (*e.g.*, in the production of diphtheria antitoxin) will be just as susceptible to tetanus toxin as a normal animal. In a very few cases the law does not hold. The only well-authenticated example of this sort is the antagonism which animals display to anthrax after injection with the products of *B. pyocyaneus*.

These preliminary considerations will serve to show the more important criteria by which the nature of a bacterial product may be determined, and its nature as a true toxin established.

These toxins were soon found to fall into two main groups—the extracellular or soluble toxins, or, as we shall call them, the *exotoxins*, and the intracellular insoluble toxins, or *endotoxins*. We shall consider these substances in turn.

### THE EXOTOXINS.

The *exotoxins* are substances which are given off in a free state when the bacteria are grown in a suitable culture medium outside the body, and can usually be separated by simple filtration (through a Pasteur or Berkefeld filter) from the organisms which produce them. We may consider them provisionally as the specific secretions or excretions of the bacteria. They are not formed by all pathogenic bacteria—that is, in the present state of bacteriological science no suitable culture media have been found in which certain organisms will produce a soluble toxin. The three most important organisms which do so are the *B. tetani*, *B. diphtheriæ*, and the *B. botulismus*. These toxins, the first two especially, are substances of the greatest interest, since they have been submitted to a most profound examination, and our knowledge of the structure of bacterial toxins, of their action on the body, and of the production of immunity thereto, is based almost entirely on the results thus obtained. In addition to these, there are substances which are much less toxic—if, indeed, toxic at all—and which fail to fulfil our definitions of a specific toxin, since an animal which has been immunized thereto is not necessarily immune to the organism, but which have many points in common with the true toxins, and will be considered in this connection. These are the bacterial cytolysins and hæmolysins,<sup>1</sup> substances

<sup>1</sup> Hæmolysis, or the liberation of hæmoglobin from red blood-corpuscles, may be brought about by a variety of agents, which fall under three main headings: (1) Simple chemical substances, such as distilled water, ether, acids, etc., which act osmotically, or by a direct solution of the stroma of



which have the power of dissolving living cells or red blood-corpuscles respectively from susceptible animals.

In dealing with these substances we will consider firstly their action, secondly their structure, and thirdly what has been established concerning their chemical relationships with other substances. The last is comparatively unimportant.

1. *Action of Toxins*.—The results of the injection of a toxin into a living and susceptible animal depend, in most instances, on the dose injected. If, for instance, we inject a large amount of the filtered broth in which the tetanus bacillus has been growing for a month or so, and which in consequence contains tetanus toxin, the animal (a guinea-pig, for example) will develop the rigidities, spasmodic contractions of the muscles, etc., characteristic of tetanus; and these make their appearance after an interval of some hours, during which period the animal shows no symptoms whatever of the disease. Great stress was laid at one time on the occurrence of this "latent period," since it was thought to be peculiar to the bacterial toxins (and to the similar substances of animal and vegetable origin), and to distinguish them sharply from other poisons, alkaloids, etc. This is hardly correct. It is true that in most cases of intoxication by bacterial toxins there is a latent period, but in a few it is practically absent. The most interesting example is the "Nasik" vibrio, an organism allied to that of cholera. This produces an exotoxin (though not a very powerful one in the sense that it kills in small doses), which proves fatal on intravenous injection into a rabbit after a period of ten to thirty minutes, and symptoms are produced before this. On the other hand, some of the alkaloids, and notably colchicine, display a well-marked latent period. The phenomenon, therefore, is not absolutely peculiar to, nor characteristic of, the toxins; but since it is so commonly displayed by them, it calls for some investigation. Moreover, we must assume that part at least of the incubation period of an infective disease is taken up by the latent period of the bacterial toxin, a circumstance which invests it with especial interest. Thus a horse which Madsen

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the corpuscles or of parts thereof; (2) the simple organic hæmolysins, which include the bacterial hæmolysins dealt with above, the hæmolysins of vegetable origin (such as ricin, etc.), and some of the hæmolysins of animal origin; and (3) the compound hæmolysins, all of animal origin, which will be dealt with subsequently. These groups differ profoundly in their action, and must be kept quite distinct.

was treating for the production of diphtheria antitoxin developed tetanus, and tetanus toxin was found in a sample of blood collected five days before the development of symptoms.

On diminishing the amount of the toxin which we inject, we find that the latent period becomes gradually longer, and the duration of the disease (*i.e.*, the time between the first development of symptoms of intoxication and the fatal issue) also lengthens. By diminishing the dose gradually we can find an amount which will just kill the animal in question in a given number of days, and, provided the test animals used are approximately the same in age and weight, we shall find that this amount, the "minimal lethal dose," is fairly constant for animals of the same species. Thus, in the standardization of diphtheria antitoxin the first step is the estimation of the minimal lethal dose of the toxin, and for this purpose it is customary to use guinea-pigs weighing from 250 to 280 grammes, and to fix a time-limit of four days. It is found that the minimum lethal dose is the same, within close limits, for all test animals, and that if a series similar in size and weight be inoculated with the same dose, the majority will die within a few hours of one another. This fact enables diphtheria antitoxin to be titrated with some approach to chemical accuracy, the test guinea-pig being used as the indicator.

On reducing the dose still further, we find that the incubation period is still further prolonged, that the symptoms are less severe, and that death may not take place, or only do so at a later period than that which has been fixed for the minimal lethal dose. Thus, in antitoxin-testing a dose of toxin which does not kill in five days is regarded as a sublethal dose, although death may take place at a later date—perhaps much later.

On giving still smaller doses the symptoms take still longer to develop, are still slighter, and are followed by recovery, and the animal may then present a certain degree of immunity to the toxin and to the organism producing it. On the other hand, under certain circumstances it may be more than usually sensitive to the action of the toxin in question.

These phenomena present some points of comparison with those which are presented in the action of the soluble enzymes, such as pepsin. In each case an excessively minute amount of the active substance will produce the given effect, and in each the effect is more rapid if a larger amount be used. In either case there is a latent period of longer or shorter duration before

the peculiar chemical action is manifested. There are several other analogies between the soluble enzymes and the exotoxins.

(a) The soluble enzymes are, without exception, all produced by living animal or vegetable cells, and are either secreted or excreted by them, or remain locked in their protoplasm. The bacterial toxins, in the same way, are all formed and eliminated by living bacteria; or, in the case of the endotoxins, retained in the cell. In other words, both extracellular enzymes and exotoxins are products of metabolism given off during the life of a living organism. Further, both substances represent a method in which the organism attempts to modify its environment and render it more suitable: the animal secretes pepsin into its stomach in order to modify the ingested proteids and render them suitable for food, and the tetanus bacillus produces toxin in a living animal because it is in itself but little adapted to grow in living tissues, but can do so easily when these tissues have been injured by the action of toxin. The spores of tetanus which have been washed free from all traces of toxin have no power of producing tetanus when injected into an animal, and are rapidly taken up by the leucocytes, or otherwise dealt with by the tissues; but if a minute amount of toxin be injected at the same time the bacteria can resist the leucocytes and tissues, which are injured thereby, and continue to grow and produce fresh toxin, giving rise to fatal tetanus.

(b) It is capable of proof that enzymes commence their action on the substances which they attack by forming a combination therewith. Thus the first effect of the addition of pepsin to fibrin is the formation of a compound between the two substances, as shown by the fact that, if the fibrin be thoroughly washed at a temperature near the freezing-point until all traces of free enzyme are washed away, it will still undergo digestion when raised to the body temperature. Further, the enzyme is less easily destroyed by heat when it has combined with the fibrin.

In a similar way it is capable of proof that the toxins unite chemically with the cells of susceptible animals. The proof may be deduced from the fact that if toxin be injected intravenously into a susceptible animal it rapidly disappears from the blood, although it does not escape, or only to a very small extent, in the secretions. When the injection is made into insusceptible animals it may disappear by a process to be discussed subsequently, or may persist for long periods. Thus in one case Metchnikoff

was able to demonstrate the presence of tetanus toxin in the tortoise, which is insusceptible to the action of that substance, at a period of *four months* after the injection. That the disappearance which occurs in susceptible animals is actually due to a combination of the toxin with the tissues of the body, and not to its destruction or elimination, is shown by the fact that the tissus of an animal which has been injected with tetanus toxin, but which no longer contains that substance in the blood, may produce tetanus when injected into a susceptible animal. In the case of fowls it seems that this power of combining with tetanus toxin is most marked in the leucocytes. Again, it is possible to reproduce the absorption of tetanus toxin by fresh tissues *in vitro*. This has been especially studied by Ignowtowsky, who showed that emulsions of liver, kidney, spleen, etc., have the power to absorb tetanus toxin, but that the subsequent injection of these cells will produce the symptoms of the disease.

It ought to follow logically that the toxin will combine *especially* with those cells and tissues which are acted upon by it in the living body, and in all probability this is the case. The proof, however, is somewhat difficult. Wassermann apparently proved the point by his demonstration of the fact that tetanus toxin is absorbed *and neutralized* by an emulsion of the central nervous system, and not by that of any other organ, although, as has been mentioned above, it is absorbed by other tissues. Now, tetanus toxin acts entirely, or almost entirely, on the central nervous system, and this well-known and oft-quoted experiment appears to constitute a proof of the point at issue. The exact interpretation of Wassermann's experiment appears, however, to be doubtful, and it is hardly safe to rely on it as a proof of the point.

With the bacterial hæmolysins, which, although of feeble toxicity, are in every other respect identical with the exotoxins, we are on surer ground. A filtered broth culture of the tetanus bacillus contains the specific toxin (tetanospasmin), and in addition a second substance, which has the power of dissolving red blood-corpuscles when kept at a temperature near that of the body. At a low temperature they do not act in this way; but if red corpuscles be added in suitable amount to a solution of tetanolysin at a temperature of 0° C. and centrifugalized, the supernatant fluid has no longer the power of producing hæmolysis. On the other hand, the red corpuscles, even after washing with normal

saline solution to remove all traces of free hæmolysin, are dissolved when raised to the body temperature. In other words, the specific hæmolysin of tetanus can form a combination with the structures on which they act. Numerous similar examples will be met with.

(c) In some of the specific exotoxins, notably that of tetanus, we meet with a similar dependence on a suitable temperature for the development of their toxic action, a property in which again they resemble the soluble enzymes. The most striking example is obtained by a study of the action of tetanus toxin on the frog, which, in common with all cold-blooded animals, is but slightly susceptible to its action. If, however, the frogs be kept in an elevated temperature— $30^{\circ}$  C. or higher—they develop the typical symptoms of the disease after five days or thereabouts. Now Morgenroth has shown that the toxin unites with the central nervous system at a low temperature ( $8^{\circ}$  C.), but without the development of symptoms. For the production of these a high temperature is necessary, exactly as in the case of the combination of tetanolysin with red blood-corpuscles and the solution of the latter.

(d) These and similar researches lead us to distinguish between two faculties of a toxin—that of combining and that of injuring; and the fact that in some instances these processes can take place at different temperatures leads us to the belief that they are quite different properties of the toxin. In other words, the mere union of a toxin with a cell is not sufficient to cause injury to the latter. This is susceptible of proof. In Ehrlich's elaborate studies on the standardization of diphtheria antitoxin he first obtained a specimen of diphtheria antitoxin, and determined its minimum lethal dose for test guinea-pigs. For the sake of simplicity we will suppose that for a given sample of toxin this was  $\frac{1}{100}$  c.c.—*i.e.*, that amount of the filtered broth culture of the diphtheria bacillus would just kill a guinea-pig weighing 250 grammes in four days. Further, let us suppose that we have a standard sample of antitoxin of which 1 c.c. just neutralizes 1 c.c. of toxin (100 lethal doses), so that the mixture of the two causes no symptoms when injected into a test animal. Diphtheria antitoxin is a relatively stable substance, and can be preserved in a dry state, at a low temperature, for long periods if light and air are excluded. It is thus possible to re-test the sample of toxin with a precisely similar solution of antitoxin after some months. When this is done, it is

found that it will have fallen off in potency; for example, it may take  $\frac{1}{50}$  c.c. to kill a guinea-pig. It might be supposed that this was due to a complete destruction of half the toxin, but this is not the case. If it were so, we should find that to neutralize 1 c.c. (= 50 lethal doses) we should require  $\frac{1}{2}$  c.c. of antitoxin, since the latter has not altered in potency. As a matter of fact, we find that we still require 1 c.c. of antitoxin; in other words, the diminution of the toxic power of the solution has not been accompanied by a diminution in its combining capacity for antitoxin. The explanation given by Ehrlich, and fully proved by analogy with numerous other similar phenomena, is that part of the toxin has altered into a substance which retains its power of uniting with antitoxin (and, as we shall show later, with the tissue cells), but which has been deprived of its toxicity. Toxin which has undergone this change is called *toxoid*. Hæmolysin also appears to undergo a similar change into hæmolysoid, and the rapid loss of



FIG. 5.—A MOLECULE OF TOXIN WITH ITS HAPTOPHORE (a) AND TOXOPHORE (b) GROUPS.

On the right a similar molecule, which has lost its toxophore group, and become converted into toxoid.

activity which tetanolysin undergoes is very probably due to a change into that substance.

The alteration of the toxin to toxoid can be best explained by supposing that the power of entering into combination and the power of intoxication reside in two different parts—which we may regard as groups of atoms—of the molecule of toxin, and by further supposing that the combining group is a relatively stable one, and that the toxic group is easily destroyed. In the very convenient nomenclature introduced by Ehrlich, and now universally adopted, the group of atoms which has the power of entering into chemical combination with the living protoplas or with antitoxin is called the *haptophore group*, whilst the portion on which the toxic action depends is called the *toxophore group*. The change of toxin into toxoid, or of hæmolysin into hæmolysoid, consists in a destruction of the toxophore group, with retention of the more stable haptophore group (Fig. 5.) From what has been said as to the dependence of the phenomena of intoxication on a

temperature approaching that of the body, it follows that the haptophore group can functionate at a low temperature ( $0^{\circ}$  to  $10^{\circ}$  C.), while the toxophore group can only do so at a fairly high one. Looked at in this way, the process of intoxication with an endotoxin, or of hæmolysis with a bacterial hæmolysin, may be divided into two stages: in the first place, the haptophore group of the toxin or hæmolysin combines with the protoplasm or with the stroma of the red corpuscle, and in the second the toxophore group exerts its action, and the cell is poisoned or the red corpuscle dissolved. The phenomena of tetanus in frogs is thus readily explicable.

We shall see several other examples of substances in which it is possible to distinguish between a combining and an active group, and the same terminology will be adopted throughout (agglutinoids, complementoids, etc.).

The change of toxin into toxoid takes place in all exotoxins, but at very different rapidities. Tetanolysin is transformed completely into hæmolysoid in a day or so, whilst tetanospasmin, the true toxin of the disease, is much more stable. The process is accelerated by heat, light, and the access of oxygen, and by certain chemical substances which are not sufficiently powerful to destroy the toxin outright. Of these the most important are a solution of iodine in iodide of potassium, and bisulphide of carbon.

The exotoxins are destroyed outright by heating to the boiling-point (to this rule there are a few exceptions, none of which has been fully examined), by strong acids and alkalis, and by the action of the digestive enzymes. They are, as a rule, precipitated by the substances which precipitate proteids, and destroyed by the substances that destroy those bodies. They have, further, the power of attaching themselves to precipitates, of whatever nature, which are thrown down in fluids containing them; so that formerly they were thought to be albumins, albumoses, nucleo-albumins, etc., since they were carried down mechanically when these substances were precipitated from a bacterial culture in which they were present along with the exotoxin. In these points again they closely resemble the enzymes.

They are substances the molecules of which must be small in comparison with those of the coagulable proteids, since they readily pass through filters (of unglazed porcelain permeated with gelatin) which retain the latter. This fact was put to an ingenious

use by Martin and Cherry in their demonstration that diphtheria toxin and antitoxin combine chemically.

Enzymes are also substances of small molecule, and pass through similar filters. When injected into suitable animals enzymes give rise to the production of anti-enzymes, which are exactly equivalent to antitoxins. Thus we see that in many points the process of intoxication with the bacterial exotoxins presents close analogies with the destruction of proteids, etc., by enzymes; and to these we might add the suggestion that it is very probable that these exotoxins act, partly at least, by a process of hydrolysis. This suggestion is based partially on the fact that the process of hæmolysis is almost certainly one of hydrolysis, and partially on the appearance of poisoned cells, which look as if they had absorbed water and became partly dissolved.

There is, however, one feature in which the exotoxins and their allies, the bacterial hæmolysins, are absolutely different from the enzymes. In the case of the enzymes a molecule attaches itself to the substance to be attacked, water is absorbed, and the whole complex molecule breaks down; and in this process the molecule of enzyme is set free, and is again ready to attack another molecule. Thus a very small amount of the active substance can decompose a large amount of fermentable substance. The toxins do not behave in this way, and, as far as we know, a molecule of toxin which has united with one molecule of protoplasm is never set free to attack another.<sup>1</sup> The proof of this is not very direct, and rests mainly on the fact that the amount of toxin necessary to kill two animals of *the same species* varies roughly with their weight. Thus the minimal lethal dose of diphtheria toxin for a guinea-pig of 250 grammes will not kill one of 400. If the molecule of toxin could attack one molecule of cell substance after another in the same way as an enzyme, we should expect it to do so, though after a longer interval. It must be confessed, however, that this proof is not very striking; exceptions frequently occur, since, as a rule, older animals are less susceptible than younger ones in proportion to the body-weight. But it is certainly true with regard to the bacterial hæmolysins, since we can test them on the same sample of blood, and when

<sup>1</sup> It may possibly undergo *dissociation*, and be set free to attack another molecule, but this is a different process: the molecule first attacked is not injured.



this is done we find they obey the law of multiple proportions with great accuracy. Thus the exotoxins differ from the enzymes mainly in the fact that each molecule of the former acts once, and once only. We shall subsequently meet another group of substances, of very similar nature but of animal origin, which have an enzyme-like action, but are destroyed in the process. They are the complements (alexins, etc.), which resemble the exotoxins in many respects, and might well be called the animal toxins.

On investigating more closely the action of the exotoxins, we find that certain of them exert their pathogenic action mainly on certain cells of the body. The most marked example of this is in tetanus, which practically only affects the cells of the central nervous system, causing in them definite histological changes, and having a pharmacological action almost exactly like that of strychnine. In the case of diphtheria also the action is most marked on these cells; this is best shown by the occurrence of diphtheritic paralysis (associated with histological changes in the ganglion cells similar to those of tetanus, and subsequent degeneration of the nerves), which occurs after the action of minute doses of the toxin.<sup>1</sup> We may fairly assume that when but an excessively small amount of toxin is present, it will unite with the cells with which it has most affinity—in this case with those of the central nervous ganglia. But diphtheria toxin is not limited in its action, as tetanus toxin is, and can act upon the tissue cells almost without exception. Thus we find that the injection of a large dose of toxin subcutaneously is followed by the production of an acute inflammatory swelling, showing that it can poison the connective tissues, and after death there may be focal necrosis of the liver, degenerative changes in the renal epithelium, fatty degeneration of the heart, etc., showing that the toxin may act on all these organs and tissues. We may regard it as a good example of a general protoplasmic toxin having, as is so frequently the case, a special action on certain cells. The toxins of most diseases come under this heading, the specialized action of the tetanus toxin being unique.

In some cases we can study the action of the exotoxins and allied substances on isolated cells *in vitro*, and these are of especial interest from the ease with which they can be investigated, and are of some importance in disease. They are the leucolysins, or leucotoxins, and the hæmolysins.

<sup>1</sup> If we accept Arrhenius's view of the interaction of toxin and antitoxin.

The leucolysins are substances which are formed by bacteria, and which have the power of killing and dissolving, or partially dissolving, the leucocytes of susceptible animals. Owing to the comparative difficulty of obtaining emulsions of living leucocytes, they have not been submitted to the same thorough examination as have been the bacterial hæmolysins; but the important relations between the leucocytes and immunity lead us to believe that they are of very considerable pathological interest. The first to be described was that formed by the *Streptococcus pyogenes*, the action of which on the living leucocytes was shown by an ingenious experiment to occur *in vitro*, and to be neutralized by means of antileucolysin, this being one of the earliest proofs that toxin and antitoxin form a chemical combination, and that the preventive and curative effects of the latter are not due to some profound influence on the tissues of the living body, by which they are rendered immune before the toxin can attack them. The method, invented by Neisser and Wechsberg, is based on the fact that living leucocytes have the power of deoxidizing and bleaching a solution of methylene blue. When a solution of the products of growth of streptococci is added to an emulsion of living leucocytes, together with a little of the dye, and a layer of liquid paraffin added to prevent the further access of air and subsequent oxidation of the methylene blue, the colour no longer disappears, showing that the leucocytes have been killed. If, however, a suitable amount of antileucolysin (obtained by injecting the filtered products of the streptococci into an animal) be added to the mixture the colour disappears, showing that the leucocytes have been protected from the action of the leucolysin, which has now been neutralized by the serum.

The action of the leucolysins can also be studied microscopically *in vitro*, when the cells are seen to become more transparent, and their nuclei to become more indistinct, and ultimately to disappear. The dissolving leucocytes look very much like those found in pus.

Leucolysins are formed by the *Streptococcus pyogenes*, the staphylococcus, and *B. pyocyaneus*, and probably by other organisms.

The bacterial hæmolysins are an interesting group of substances which are closely allied to the exotoxins in their reactions, but are little toxic, if at all. The most toxic appears to be that of *Streptococcus pyogenes*, to which some observers, though not all, attribute feeble poisonous powers when injected into animals.

At the same time, it is quite certain that these substances play some part in the production of the symptoms of various diseases. The anæmia which develops so rapidly in acute sepsis is well known, and is one of the most constant symptoms of that affection; it is to be ascribed, in part at least, to the destruction of the red corpuscles by the hæmolysins elaborated by the streptococci, staphylococci, or colon bacillus, if these happen to be the infective organisms. The blood of an animal which has been injected with virulent streptococci is found to contain hæmolysin, and that this is actually the hæmolysin produced by the streptococcus is shown by the fact that the action of this serum is restrained by the addition of serum from an animal treated by injections of streptococcic hæmolysins. Thus it is proved that this organism elaborates its hæmolysin *in vivo* as well as *in vitro*; and several observers have found that it is those species of streptococcus which are specially virulent to animals and man that form hæmolysins, the harmless ones doing so to a small extent, if at all. The same is true for staphylolysins. Further, when a culture of streptococci which is but slightly virulent and forms but little hæmolysin is rendered more virulent by "passage" through rabbits, its power of forming streptocolysin is increased.

These facts render it certain that some at least of the bacterial hæmolysins act, to some extent, as exotoxins, though the organisms producing them certainly form other and more important specific poisons. We may consider them as accessory toxins of comparatively little pathological importance.

The similarity in nature of the bacterial hæmolysins and the specific exotoxins is shown by the fact that (in the case of streptocolysin, and probably in others) they can become converted into *hæmolysoids*, analogous to toxoids. This is shown as follows: Streptocolysin becomes inert in a week. If a small quantity of blood-corpuscles be added to an excess of this inert solution, and then thoroughly washed and added to a fresh and active solution of streptocolysins, they will not be dissolved; the corpuscles had evidently become saturated with inert hæmolysoid, and are now unable to take up any hæmolysin, their combining powers being satisfied (see Fig. 6).

The chief bacterial hæmolysins are those formed by the tetanus bacillus, the staphylococcus, the *Streptococcus pyogenes*, the *B. pyocyaneus*, *B. coli*, and the typhoid bacillus. Their more important features will be recapitulated briefly.

Tetanolysin is formed along with the specific toxin, tetanospasmin, when the *B. tetani* is grown in broth, the two substances being formed in variable amounts under different circumstances. It is very unstable, disappearing entirely in a day or two at the room temperature, and being destroyed by heating to 50° C. for twenty minutes. It cannot be obtained free from tetanospasmin, but a solution of tetanus toxin can be deprived of its lysin, and only the specific toxin left, by adding some red corpuscles to the solution, kept at a low temperature, and centrifugalizing them

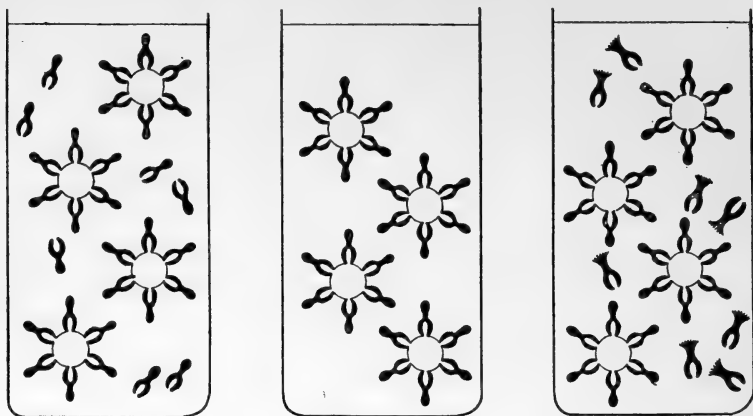


FIG. 6.—A "SATURATION EXPERIMENT" SHOWING THAT HÆMOLYSOID HAS THE POWER OF COMBINING WITH RED BLOOD-CORPUSCLES, AND SHIELDING THEM FROM THE ACTION OF HÆMOLYSIN. (SCHEMATIC.)

In the first tube the corpuscles are shown in presence of an excess of old or heated hæmolysin; in the second they are washed clear from this excess, and are apparently unaltered; in the third active hæmolysin is added, but the corpuscles are not dissolved, as they would be in a control-tube with normal corpuscles.

off; the supernatural fluid will contain tetanospasmin, whilst the tetanolysin will have combined with the corpuscles.

Staphylolysin appears in alkaline cultures on the fourth day, and reaches its maximum between the tenth and twelfth. It is an unstable substance, but more stable than tetanolysin, persisting for a fortnight at the room temperature, and requiring a temperature of 56° C. for twenty minutes for its complete destruction—and in this case the destruction appears to be really complete, for the injection of the heated solution is said not to lead to the production of an antistaphylolysin. Many normal sera, especially those of man and the horse, contain antistaphylolysin;

perhaps this is the reason why slight staphylococcic infections in man are not associated with marked hæmolysis.

Streptocolysin is formed in forty-eight hours when a virulent streptococcus is incubated in broth containing blood-serum or ascitic fluid, and it is a remarkable fact that the nature of the serum used modifies the lysin produced. Thus if ox serum be employed the lysin will act on the corpuscles of the guinea-pig, rabbit, and man, but not those of the ox or sheep; whilst all these will be dissolved by that grown in broth to which human serum has been added.

Streptocolysin is less thermolabile than tetanolysin and staphylo-lysin, requiring an exposure of ten hours to  $55^{\circ}$  C. or of two hours to  $70^{\circ}$  C. for complete destruction.

The other bacterial hæmolysins—*i.e.*, those produced by the *B. pyocyaneus*, *B. typhosus*, and *B. coli*—are quite different from the foregoing in being thermostable. Thus pyocyanolysin resists a temperature of  $120^{\circ}$  C. for thirty minutes. Typholysin appears to be less resistant, but is definitely thermostable. Colilysin is as stable as pyocyanolysin; it is not destroyed by a temperature of  $120^{\circ}$  C. for half an hour, and does not undergo spontaneous weakening for months. It is obvious that these substances are different in character from the other hæmolysins and exotoxins, and the fact that (in the case of pyocyanolysin and colilysin, the most heat-resistant of the group) the hæmolytic property of the culture only appears when it becomes strongly alkaline and is roughly parallel in degree to the amount of alkalinity, has led some to think that the substances are not the true hæmolysins at all, but merely simple alkaline chemical products of growth; and this is corroborated by the fact that much of the hæmolytic power is taken away on neutralization with a weak acid. It appears that this is not the case, since in a culture of *B. coli* at a temperature of  $23^{\circ}$  C., the alkalinity reaches its maximum on the fifth day, whilst the hæmolytic property does not appear until later. The subject requires further investigation, and at present it is advisable to disregard these substances which differ so much from their allies.

The chemical nature of the exotoxins has been the subject of much controversy, and is still very imperfectly understood. It will not be discussed at great length, since from the point of view of immunity it is not of very great importance.

The close analogy between the bacterial exotoxins and certain

vegetable toxins, such as ricin and abrin (which were thought to be definitely proteid in nature), led, very early in the history of the subject, to the view that these toxins are proteid in nature, and this view was strengthened by the fact that when the diphtheria or tetanus bacillus is grown in an albuminous fluid, proteid substances which are toxic and give the specific reactions of the toxins in question can be precipitated therefrom. Thus Hankin and Sidney Martin found toxic albumoses in bacterial cultures, and apparently succeeded in proving that abrin is an albumose. Brieger isolated a toxalbumin from diphtheria cultures, and Sidney Martin showed that from cultures of the same organism in alkali albumin it is possible to prepare an albumose which he thought to be the specific toxin. Many similar researches were published, and the exotoxins were regarded as being albuminoid in nature, and the term toxoprotein was applied to them. Several writers—Duclaux in particular—argued that this was not the case, and thought that these proteid substances merely carried the true toxins with them mechanically on precipitation, just as the precipitates of inert substances such as cholesterin will carry enzymes down with them. This theory was supported by Brieger and Cohn, who purified tetanus toxin from all ordinary proteids, and especially by the researches of Buchner and Uschinsky, who cultivated tetanus and diphtheria bacilli in solutions devoid of all albuminous material, the necessary nitrogenous nutriment being provided by asparagin. Under these circumstances the toxic solution contains neither albumoses, peptones, nor known proteids of any description. The toxins thus formed are present in infinitesimally small amount, and have never been obtained in a pure form, nor submitted to ultimate analysis. It is known, however, that they contain nitrogen, that they are readily destroyed by heat, and that they are dialysable. These considerations lead us to the supposition that they are closely allied to the proteids, and especially to the albumoses or peptones, but form a group differing from any of them, and approximating more closely to the enzymes. That this is the case appears certain from the facts brought out by researches on the antibodies; all the substances of *known* chemical composition which lead to the production of antibodies on injection into suitable animals are either proteids or else substances of indefinite composition similar to the toxins, and apparently all proteids will lead to the production of antibodies on injection into suitable

animals. These facts lead us to the belief that the exotoxins are, at any rate, allied to the proteids, and form with the enzymes a group of the substances of peculiar composition.

We have referred above to ricin as a substance once thought to be of definite proteid nature, and a few facts may be given concerning this substance, which is closely allied in every way to the bacterial toxins, and which may be taken as a type of the vegetable toxins or phytotoxins. It occurs in the seeds of various species of *Ricinus*, and was formerly regarded as being a proteid, since, like the bacterial toxins, it is carried down mechanically with proteid precipitates. Thus Stillmarck regarded it as a globulin, since he prepared it from the seeds by a process which was adapted to the separation of those substances (solution in 10 per cent. NaCl, precipitation with sodium or magnesium sulphate, and dialysis). But Jacoby thought he had succeeded in separating it entirely from its proteid accompaniment, making use of the fact that when a mixture of ricin and the other substances present in the seeds are acted on by trypsin, the active principle is acted on but slightly, if at all; the ricin itself, in a pure state, is readily digested by trypsin, like the other toxins. Jacoby digested an extract of castor-oil seeds for five weeks, and then added enough ammonium sulphate to render the fluid 60 per cent. saturated, and ricin was thrown down in an almost pure state; it was purified by reprecipitation, and then found not to give any of the proteid reactions, though it retained the characteristic toxic properties of the substance. Quite recently, however, Osborne, Mendel, and Harris obtained ricin in a very pure form, and found it to be either proteid in nature or at least inseparably associated with coagulable albumin; its toxicity was removed by tryptic digestion or heat coagulation. Its great potency ( $\frac{1}{1000}$  mgr. being a lethal dose per 1 kilo of rabbit) suggests that the substance which they prepared was really pure.

Ricin resembles the bacterial toxins in the following points: It has a period of incubation; it gives rise to an antitoxin when suitably administered; it is extraordinarily potent, the lethal dose per kilo of weight (in rabbits) being a minute fraction of a gramme; it is destroyed by boiling; and it is much less potent on ingestion than on injection. Its main toxic properties are fever, loss of weight, albuminuria, hæmaturia, and hæmorrhage from the intestine; death occurs in about twenty-four hours with acute nervous symptoms. It has a most interesting and characteristic

action on the blood, clumping the corpuscles in a peculiar way, even at a dilution of 1 : 600,000, and also hæmolyzing them.

Further research leads us to believe that the toxin molecule may be, and under ordinary circumstances is, actually of more complex constitution, being combined with a molecule of true proteid. We have already pointed out the fact that streptocolysin differs in its reactions according to the origin of the serum on which it is grown. The best example, however, is derived from diphtheria toxin when grown in broth containing blood-serum or plasma, and subsequently heated. This solution is but feebly toxic, probably from the toxins having undergone a change into toxoids, yet it possesses the power of immunizing an animal against diphtheria, and of stimulating the production of antitoxin to an unusual degree, but only on condition that it is injected into an animal of the same species as that from which the serum in which the bacillus grew was obtained. Thus horse-serum toxin will stimulate the production of antitoxin in horses, but not in goats or rabbits, and so forth. We are justified in supposing that the essential toxin molecule formed by the diphtheria bacillus exists in this fluid in a state of combination with a specific proteid of horse serum, and that the resulting compound molecule differs from that form when the bacillus is grown in goat serum, in which the essential toxic molecule is united with a different proteid. We may suppose that this essential toxin molecule is produced in Buchner and Uschinsky's asparagin solution, but that it is not produced under ordinary conditions, being in a state of combination with proteid materials of more complex structure. These facts render further research into the chemical nature of the exotoxins of comparatively little importance.

#### THE ENDOTOXINS.

In the case of diphtheria and tetanus and a few other organisms the mode of formation of toxins is a perfectly simple one, and one exactly analogous to the formation of soluble enzymes. In most other cases, however, the facts are less easy to understand, and seem to point to the formation of a toxin which remains under normal circumstances locked up in the substance of the bacteria, just as invertase and diastase are contained within the yeast cell, and not excreted by it into the surrounding fluid. A satisfactory theory as to the nature of these toxins is not forthcoming, and the experimental results obtained by various



observers is very contradictory and difficult to understand, the difficulty being increased by the fact that in the earlier researches the distinction between antitoxic and bacterial immunity was not understood. As a result of this we have to be careful in interpreting these early results, so as to make sure that when the author speaks of a serum as containing antitoxin he does not really mean that it contains a protective substance which may not be an antitoxin at all. In many cases the data are not sufficient for us to discover its actual nature.

The organisms on which the chief amount of experimental work has been done are those of cholera, typhoid, tubercle, anthrax, and the pneumococcus, and it is these which we shall discuss in chief, excluding, however, the consideration of the toxins of the tubercle bacillus for separate consideration in a subsequent chapter.

The general facts brought out by experiments with organisms such as those of typhoid and cholera are these: The germ-free filtrate of a young and actively growing culture is very slightly toxic, if at all. The filtrate of an older culture is usually feebly toxic, but to a degree which can hardly be compared with that of diphtheria or tetanus; it may take several cubic centimetres to kill a rabbit or guinea-pig. And even this feeble toxicity is largely discounted by the fact that the filtrate may contain acids, nitrites, etc., which are poisonous, but in no way related to true toxins. Yet in some cases exotoxins do exist in the filtrate, since it is possible to obtain an antitoxin for them. The reactions of these antitoxins, however, are peculiar, in that the law of multiples does not seem to apply beyond a certain figure. This is well seen in the case of *B. pyocyaneus*, which forms a sort of connecting-link between cholera and diphtheria, in that it forms a definite though feeble exotoxin, whilst the immunity to it is bactericidal. Wassermann showed that it is possible to produce a true antitoxin against this toxin, and to determine the amount which will just neutralize one lethal dose. He found, however, that a multiple of this amount of antitoxin beyond ten would not protect an animal against a corresponding dose of toxin. With larger doses of toxin even a great excess of antitoxin was powerless to prevent a lethal issue. Similar results have been obtained in the case of cholera. These and other results have led some authorities to consider that these exotoxins are not the specific toxins which the pathogenic action of the bacillus defends, but

secondary products of but little importance. Thus, in the case of *B. pyocyaneus* it is possible to immunize an animal by cautious injections of living organisms, yet its serum has no antitoxic powers against the so-called toxin.

These facts have turned attention to the bodies of the bacteria themselves, with the result that they have been found to be definitely toxic, although in many cases the toxicity is not great. The theory has therefore been put forward—by Pfeiffer especially—that under normal circumstances these organisms do not secrete a soluble toxin, but that their protoplasm itself is toxic, and that it is only set free on the death and solution of the cell, thus accounting for the slight toxicity of old cultures, in which such a solution of the cells must take place. The symptom of the disease caused by these organisms is attributed to the solution of the bacteria by the fluids of the body.

The study of these endotoxins has not left the matter clear. They are present in the bodies of the bacteria, whether the latter have been killed by heat, by antiseptics, or by drying. They are apparently but slightly soluble in water, but can be obtained in solution by autolysis of the bacteria in normal saline solution in the incubator, by grinding the dead bacilli, or by the use of very high pressure (the method introduced by Buchner for the extraction of endo-enzymes from yeast). But it did not appear possible to produce an antitoxin against this poisonous material; in addition, animals which have been immunized against the living organism might be as susceptible as before to the dead bacteria, or to extracts of them.

The researches of Macfadyen and Rowland have apparently disproved this, and tend to support the opinion that the endotoxin is a true toxin, for which an antitoxin can be obtained. They obtained young cultures of various organisms, froze them at the temperature of liquid air, and then ground them (whilst solid) into an impalpable powder. This was made into a paste with normal saline solution and centrifugalized, to remove any solid particles. The juice thus obtained was sterile. It was more powerful than endotoxins prepared in other ways, and it acted very quickly, having a very short period of incubation, if any. Thus, in the case of the typhoid toxin 1 c.c. killed in three hours and  $\frac{1}{50}$  c.c. in less than two days, on intraperitoneal injection. It was less active on subcutaneous injection—not more so, in fact, than other toxins of the typhoid bacillus—requiring

$\frac{1}{2}$  to  $\frac{1}{10}$  c.c. to kill in seven days. Macfadyen and Rowland found that they could immunize animals against their toxin, and that its serum was antitoxic. These researches are difficult to harmonize with those of other observers. We must admit, however, that it is possible to prepare an antitoxin to the endotoxins. The failure of other observers to do so may be owing to the fact that their toxins were not prepared in so suitable a manner for this purpose, and may have undergone some unknown secondary alterations.

But these researches do not clear up the whole of the mystery, for some observations of Metchnikoff and others show that the *V. cholerae* can produce a soluble toxin whilst in the animal, and apparently without being killed in the process. These observers prepared collodion bags, which they filled with cultures of this organism, hermetically sealed, and inserted into the peritoneal cavities of guinea-pigs. The animals died in a few days with the symptoms of cholera intoxication, although no bacteria had escaped from the sacs; the organisms in that situation were still alive. Control experiments with dead organisms showed that little toxin was present; the animals remained alive, though they might show some symptoms of toxic action. It appears, therefore, that the living bacteria do elaborate an exotoxin whilst within the animal body, and that this exotoxin has the power of diffusing through a collodion membrane. Welch has suggested an explanation which cannot be discussed fully here, but which may be mentioned briefly. He points out that when bacteria are injected in living animals the tissues of the latter react and produce substances—bacteriolysins, etc.—which are injurious to the bacteria, and which determine in part the resistance of the host, and suggests that the bacteria may also react in a similar way to the cells with which they are brought in contact. Just as the animal host only produces its toxins—the bactericidal substances—when the bacteria are brought into contact with it, so the bacteria may only produce their protective substances—the unidentified true toxins—when brought into contact with aggressive animal cells. If this is the case it is obvious that we cannot expect to produce these toxins *in vitro*, except perhaps by cultivation of the bacteria in question in fresh serum from an immunized animal.

### CHAPTER III

## THE PHENOMENA OF ANTITOXIN FORMATION

As a general rule, to which there are important exceptions, it is necessary to make use of susceptible animals for the production of antitoxin. When toxin is injected into animals in which it produces no injurious effects, it either disappears rapidly from the blood or remains for a long time in that fluid or in the tissues without leading to the formation of antitoxin. The most remarkable exception to this rule is the way the cayman reacts to tetanus toxin. The animal is immune, and if kept in the cold ( $20^{\circ}$  C.) the toxin soon disappears from the blood, no antitoxin being formed. If, however, it is kept at an elevated temperature ( $32^{\circ}$  to  $37^{\circ}$  C.), the toxin disappears as before, but now antitoxin makes its appearance (Metchnikoff). Such cases are exceptional, and when we wish to procure antitoxin, we make use of an animal in which the toxin in question produces symptoms of intoxication. The process is usually much easier in large animals, such as horses or goats, than in small ones, such as rabbits or guinea-pigs, the immunization of which presents considerable difficulties. We shall take as illustrations of the general phenomena of the process the methods adopted for procuring diphtheria antitoxin and tetanus antitoxin from horses, since these have become so familiar from their extensive application.

On injecting a small dose of a potent diphtheria toxin subcutaneously into a horse—say,  $\frac{1}{2}$  c.c.—under the skin of the neck we find there is a latent interval of a few hours or a day before the development of symptoms; then there is a *local reaction*, consisting in the formation of a hard brawny mass of inflammatory oedema round the site of the inoculation, and a *general reaction*, consisting in fever, anorexia, and symptoms of general malaise. These symptoms last a day or two, according to the dose of toxin injected, its potency, and the degree of sus-

ceptibility of the animal; and when they have passed off a small amount of antitoxin will be found in the blood, and the animal will, as a rule, be found to be less susceptible to the action of the toxin than before, so that the injection of the same dose will produce less reaction, both local and general.

This, however, is not always the case, and careful research leads us to the belief that the appearance of immunity is preceded by a period of *hypersensitiveness*, in which the animal betrays a greatly increased susceptibility to the action of the toxin, and this in spite of the fact that it may contain quite large quantities of antitoxin in its blood. Thus it happens not infrequently that after a horse has passed successfully through the early stages of immunization to diphtheria toxin, and has developed far more antitoxin than is necessary to neutralize the doses of toxin with which it is being treated, it yet will die after the injection of an amount which it would appear must be immediately rendered inert as soon as it came into contact with the plasma. Such cases have been reported from the Pasteur Institute, Behring and Kitashima, and others, and by Brieger for tetanus. In the latter an immunized horse died after an injection of tetanus toxin with the typical symptoms of tetanus intoxication, and after death its blood contained much free antitoxin. The phenomenon has probably been witnessed by most observers who have been engaged in the manufacture of antitoxin, though it has become much less frequent since the introduction of modern methods for the early treatment of animals. It is an exceedingly puzzling one, and we shall leave its further interpretation until later; here it is sufficient to say that Behring's theory of the occurrence of a stage in which the tissues are hypersensitive to the toxin is well established.

The difficulty of immunizing the small animals of the laboratory to these toxins appears to depend in large measure on the marked development of hypersensitiveness. Thus Behring and Kitashima found that they could kill a guinea-pig with  $\frac{1}{400}$  of the "minimal lethal dose" of tetanus toxin, if this amount were divided into several doses and given at suitable intervals, and similar facts have been recorded by others.

The most striking proof of the occurrence of hypersensitiveness in the process of immunization has been investigated by Behring, who pointed out that normal horses show no local effects from the injection of small quantities of tetanus toxin; their connective tissues are insusceptible to its action. As the animal becomes

immunized to the action of the toxin this is not the case; the tissues at the site of inoculation react to the poison with the production of a mass of inflammatory oedema similar to that seen in a horse injected with diphtheria toxin. It is obvious that these connective tissues have become more susceptible to the action of the tetanus toxin, and this in spite of the antitoxin with which they are bathed.

In order to avoid the difficulties arising from the occurrence of hypersensitiveness in the early stages of immunization, the use of unaltered toxin has now been practically abandoned, the following methods, either alone or in combination, being employed instead :

1. The use of mixtures of toxin and antitoxin, the latter being present in amount sufficient to neutralize all the toxin, or in excess. This is repeated several times, the amount of antitoxin given being gradually reduced, until at last a small amount of unaltered toxin is given.

It must not be thought that the immunity which is acquired in this case is simply passive, and due to the free antitoxin which is injected. The process is probably fundamentally different. We shall revert to it subsequently.

2. The injection of *toxoids*. This method is of especial advantage in the case of tetanus, to which toxin animals are extremely sensitive, and the dangers of the early stages of the process of immunization are very great. The toxin formed in the cultures may be transformed into toxoids by the action of trichloride of iodine, a solution of iodine in iodide of potassium, or by heat, the filtered cultures being exposed to a temperature of 60° C. for a time sufficient to destroy their toxicity. Toxin that has been heated to a temperature much higher than this is completely destroyed, and is useless for the process.

3. The use of *serum toxin*, which probably contains toxoids in an unusual condition of activity. This method was introduced by Cartwright Wood, and is now in general use in this country for a part at least of the process of immunization, since it leads to a more rapid production of antitoxin of high potency than can be obtained by other methods in the same time. Ordinary alkaline broth is inoculated with diphtheria bacilli, and incubated for a week at 37° C. Then 15 to 30 per cent. of its volume of serum from an animal of the same species as is to be used in the process of immunization is added, and the incubation continued for a month

or six weeks. It is then heated to 65° C. for half an hour and filtered. It gives rise to marked febrile reaction and but little local reaction. The initial dose is 200 to 300 c.c.

In giving these large doses the most convenient method is to use a large wash-bottle, the side of which is graduated in cubic centimetres. To the outflow arm there is attached 2 or 3 yards of pressure tubing, in the farther end of which a strong exploring needle is inserted, and firmly wired in place. The pressure is obtained by means of a bicycle pump attached to the inflow tube of the wash-bottle by means of pressure tubing. There should be a lateral branch communicating with a manometer, by which the pressure can be regulated. Very high pressure is sometimes necessary, especially in the later stages of the process, when the subcutaneous tissues of the horse's neck become sclerosed and dense from the repeated injections. The apparatus is most easily sterilized by passing strong carbolic lotion through it.

On testing the blood-serum from time to time, it is found that the amount of antitoxin gradually rises, each injection being followed by an increase in the antitoxic value of the serum. Thus the process is a cumulative one, the antitoxic level being raised step by step until a certain height is reached. This height differs in different animals. Thus Atkinson, in summarizing his experience of 100 horses, found that half of this number gave less than 300 units of antitoxin per cubic centimetre, a quarter between 300 and 500, whilst three gave more than 800. There appears to be no method of investigation by which the value of a horse as a source of antitoxin can be predicted early in the course of treatment, and the great variability amongst different animals is probably the reason that different observers have come to such divergent opinions as to the best doses to give and the most suitable intervals between each. Here are three chief methods:

(a) By the use of large doses of toxin, 250 to 500 c.c. every day, or almost every day, leaving an interval of a week or ten days before the bleeding, so as to allow the last injection to produce its maximum effect.

(b) The use of large injections (similar to the former) at longer intervals—five to ten days.

(c) The use of relatively small doses of weak toxins repeated every day.

All these methods have their advocates, and good results can apparently be obtained by all.

On ceasing to inject toxin, it usually happens that the antitoxic value of the serum commences to decline, and, in the absence of further injections, would probably continue to do so until it had entirely disappeared from the blood. In a few cases, however, a period of antitoxic equilibrium is maintained for some time, the amount of antitoxin lost by the excretions or destroyed in the system being compensated for by a fresh production of the same amount. When this is the case the phenomena resulting from the injection of a single dose of toxin can be traced with ease, and is of great importance, as will appear subsequently. The first effect of the injection is the production of a *negative phase*, in which the amount of antitoxin in the blood is suddenly and greatly diminished. This production of a negative phase is apparently a general phenomenon, and is found to occur in the development of nearly all antibodies in which it has been investigated. If the dose of the primary substance (toxin, etc.) is very small, the negative phase may be short in duration and very slight in extent, and may be overlooked, or may possibly be omitted altogether. Its explanation is very uncertain and cannot be discussed here, but it must be pointed out that it is *not* due to the neutralization of the antitoxin in the blood by the toxin injected; the proof of this is that it is large out of all proportion to the latter. Thus in one reported case the fall in antitoxic value of the serum which occurred in the negative phase would have required an injection of toxin 12,000 times as large as was actually given if it were due to simple neutralization. The length of the negative phase varies in different animals, and can only be learnt by experiment. It appears to be roughly proportional (in the same animal) to the amount of primary substance injected: the larger the doses of toxin, the greater the fall and the longer its duration. It is, of course, synchronous with the toxic symptoms, if any, of the substance injected, since both are due to the action of this substance on the blood and tissues; but the two do not appear to be mutually dependent: a well-marked negative phase may appear without any other symptoms of disease.

The negative phase is succeeded by a rise, the positive phase, in which the antitoxic value of the blood reaches and usually surpasses its previous level. It commonly reaches its maximum in about a week, and then commences to decline; hence it is



advisable that the animal should be bled for antitoxin after a rest of about a week from its last injection.

The bleedings are carried out at the laboratories of the Royal Colleges of Physicians and Surgeons in the following manner: The receptacles for the blood are 2-pound glass jam-jars, which are sterilized by heat and covered with parchment paper which has been soaked for some hours in 1 : 20 carbolic. Two layers of this are used, and the lower one has two radial slits cut in it, leaving a triangular wedge, which can be raised and access to the bottle thus obtained. Twelve or fourteen of these are required for each horse, and each is filled about two-thirds full.

The side of the horse's neck is shaved and washed with a solution of lysol, or a lysol dressing is put on an hour or two before the operation. The horse is placed in the stocks, and if violent the head is restrained by a twitch. It is then necessary to apply pressure at the lower part of the neck, in order to distend the jugular vein; this may be done by the thumb of an assistant, or, better, by means of a firm leather plug, which is pressed into the groove in front of the sterno-mastoid muscle by means of an arrangement of straps devised by Dr. Cartwright Wood. In this way the vein is temporarily occluded, and stands out clearly above the region where the pressure is applied. The operator (having sterilized his hands as for a surgical operation) then makes an incision about 2 inches long and above or just internal to the vessel; this should open the deep fascia, but need not actually expose the vein. He then takes a trocar and cannula having a diameter of about  $\frac{3}{16}$  inch, and pushes it firmly downwards into the vein; success in this is shown by the blood oozing up by the side of the trocar. An assistant now stands ready with a short metal tube which fits inside the cannula and communicates with 2 or 3 yards of indiarubber tubing, with a foot or so of glass tubing at its farther end. The whole has been sterilized by being soaked in lysol or carbolic lotion. A second assistant now reflects half of the outer parchment covers of one of the jam-pots, reflects the triangular strip which has been already cut in the inner cover, and inserts the glass tube in the opening. The operator then removes the trocar, and the first assistant rapidly fits the metal tube attached to the rubber tubing into the cannula; when this is done quickly hardly any blood escapes. The blood now passes through the rubber tubing into the jam-pot, which rapidly fills. When about two-thirds full the

assistant pinches the indiarubber tube and places the outflow tube in a second pot. The outer cover is replaced on the first pot, which is removed to a warm place to clot. The process is repeated until twelve or fourteen pots have been filled.

Horse's blood coagulates slowly, and a well-marked buffy coat is formed. In twenty-four hours this will have contracted, and much of the serum will be squeezed out. In order to draw this off use is made of a wash-bottle, the short tube of which is connected with a water-pump, such as is used for filters, by which a partial vacuum can be maintained. The long tube is connected to a piece of indiarubber tubing terminating in a length of glass tubing. A jam-pot is opened by half reflecting the outer cover and lifting the triangular strip cut in the inner one, and the glass tube is inserted. Air is now sucked out of the wash-bottle by turning the tap which puts it into communication with the suction-pump, and the serum siphons over. When all the serum has been abstracted a second jar is treated in the same way, the parchment cover of the first being replaced, and the process is continued with all the jars. In twenty-four hours more serum will have appeared, and the process is repeated, and a small amount may often be obtained on the third day. In this way the total yield of antitoxin is usually nearly 50 per cent. of the total volume of blood ( $4\frac{1}{2}$  to 5 litres).

The antitoxin thus obtained is usually sterile, the most careful precautions being taken to prevent contamination. Carbolic acid (0.3 per cent.), or trikresol (0.3 per cent.), or a mixture of the two, must now be added to preserve it. It is then filtered through a Berkefeld filter (not a Chamberland filter, through which it passes with great difficulty, if at all), a low pressure only being used, and finally tested for sterility by means of cultures, and for the presence of toxins by the injection of large (10 c.c. or more) amounts into normal guinea-pigs.

A specimen is taken at the time of the bleeding, and this is tested for antitoxic value in the manner to be described subsequently. The results of this testing will give the amount necessary to obtain the required dose, and this amount is placed in sterile tubes or bottles ready for use. In most cases mixtures are made, antitoxin of low potency being mixed with more powerful sera in order to obtain the requisite dose in a given volume of serum. An ingenious machine is used by which the tubes or bottles are filled automatically with the antitoxin in the required amounts.

In the earlier stages of immunization, as we have seen, each injection is followed (after a negative phase) by a rise in the antitoxic value of the serum above its previous level. In the stage which now follows this does not occur, or not definitely; there is a negative phase, but it is found impossible to force the antitoxic value above a certain level, which varies in different horses. This second stage, or period of maintained maximum, varies in different horses, and may last a few months or a year. While it lasts there are, of course, oscillations; it falls, for instance, if the animal contracts any disease or suffers in general health, but its general average is about the same.

Sooner or later this state of affairs changes, and the antitoxic value of the serum begins to fall, and cannot be raised or even

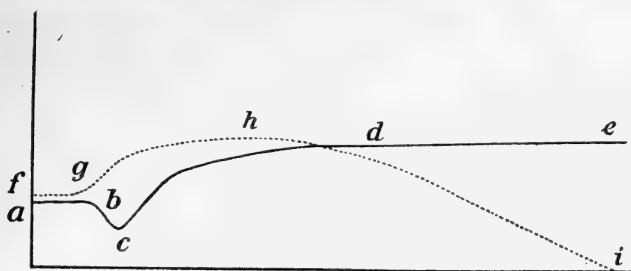


FIG. 7.

*a, b*, Normal resisting power; *b, c*, period of hypersensitiveness; *c, d*, period of rise in immunity; and *d, e*, maintained high level thereof. *f, g*, Normal amount of antitoxin; *g, h*, period in which it increases; and *h, i*, gradual fall and ultimate (theoretical) disappearance.

maintained at its former level in spite of very large doses of toxin. It trends steadily downward, although the animal may continue to give useful serum for a long time. Thus in one of Atkinson's best horses (out of a series of 100) the serum contained 1,000 to 1,100 antitoxic units per cubic centimetre for ten months, and then gradually sank, but remained above 300 units per cubic centimetre for two years.

After a prolonged rest the power of manufacturing antitoxin may return, but then only lasts a short time, and cannot be renewed again.

The third stage, therefore, consists in a gradual disappearance of antitoxin from the blood, *without any loss of the immunity* to the toxin. It would seem, indeed, as if the immunity reaches its highest level at this point, in spite of the almost complete absence

of antitoxin. Thus the two phenomena do not run *pari passu* with one another. This is illustrated in the foregoing diagram, which represents in a purely schematic way the period of immunization and utility of an antitoxin horse, the height of the continuous line from the base representing the degree of immunity, that of the dotted line the amount of antitoxin in the blood.

## CHAPTER IV

### INTERREACTIONS OF TOXIN AND ANTITOXIN

STARTING from the facts that a suitable dose of antitoxin will prevent the development of symptoms if toxin is injected shortly before, at the same time, or shortly after, or that if antitoxin and toxin be mixed *in vitro* and injected subsequently, no symptoms develop, we have to inquire the mechanism by which this is brought about. Two theories suggest themselves at once. The antitoxin might act on the cells of the living body in such a way as to render them insusceptible to the action of the poison, or, in other words, render them immune, *or* the toxin and antitoxin might unite chemically to form an inert and harmless compound. When the fundamental facts of antitoxic action were first discovered, the majority of pathologists probably inclined to the former alternative, the latter seeming too simple and teleological. A certain amount of experimental evidence was also forthcoming in favour of this view, but as this has a merely historical value it will not be considered. It is now fully proved that toxin and antitoxin form chemical compounds, and that the prophylactic and curative value of the latter is to be explained simply on the grounds that this compound is inert, or devoid of toxic action on the animal cells. The evidence in favour of the occurrence of this chemical combination requires brief discussion.

The first group of experiments pointing in this direction are those in which the toxin and antitoxin are mixed *in vitro*, and the result tested by means of red blood-corpuscles as indicators, the intervention of the cells of the living body being thus excluded. (Many of these experiments can be repeated on corpuscles which have been heated to a temperature sufficient to destroy the life of isolated body cells, and the possible objection that the corpuscles are "surviving" thus removed.)

The first of these researches was that of Ehrlich, who showed

that the agglutinative action of ricin on red blood-corpuscles could be inhibited *in vitro* by means of the serum of an immunized animal. Kanthack showed that the action of snake-venom in inhibiting the coagulation of blood was similarly prevented *in vitro* by its appropriate serum, whilst Kossel and others did the same for the hæmoglobin of eel's blood, and Ehrlich for tetanolsin. The previous cases were not of true bacterial toxins, and might possibly be open to objection on that account. The experiment of Neisser and Wechsberg on the effect of leucocidin on leucocytes *in vitro*, and its inhibition by means of an antiserum, is another case in point. It is true that in this case the leucocytes are living, but we can hardly imagine that they have become immunized by the action of the serum, or that the phenomenon can be explained on any hypothesis other than that the toxin and its antiserum have combined.

The second and most important series of researches are those of Martin and Cherry, who show that several toxins (*e.g.*, that of diphtheria and snake-venom) pass through a porcelain filter which is impregnated with gelatin, whereas their appropriate antitoxins, being composed of larger molecules, do not. (This had previously been proved by Brodie.) They found, further, that when a mixture of toxin and antitoxin was placed on such a filter the first portion of the filtrate was toxic, but that the amount diminished, and all toxicity disappeared a few minutes after the mixture had been made. The inference is clear: the toxin had united with the antitoxin to form a molecule as large as, or even larger than, that of the latter, and therefore, like it, unable to pass through the pores of the filter. These researches have been confirmed by Brodie, and form, on the whole, the most striking direct proof of the union of the two substances yet brought forward.

Calmette found that snake-venom is more heat-resistant than its antitoxin, withstanding a temperature of 80° or 90° C., whereas the latter is rendered inert at 68° C. He was then able to show that a neutral mixture of the two could be rendered toxic again by exposure to a temperature of 70° C.; and this fact was used first as an argument against the chemical theory of combination, and secondly as a proof that the toxin is not destroyed when it unites with antitoxin. As a matter of fact, neither inference is necessarily correct, and the experiment was shown by the further researches of Martin and Cherry to constitute a proof of the

chemical theory: for they found that if the mixture were allowed to stand for some time at the temperature of the body before being heated, its toxicity was not restored by a temperature of  $70^{\circ}$  C. This seems to show that the toxin did not exist as such in the mixture, otherwise it would not have been destroyed by the heat; it must, therefore, have become combined with the antitoxin, or at any rate modified by it in some way. On the other hand, the experiment does not prove that the toxin is completely destroyed beyond all power of further activity; it simply shows that, when in a condition of combination with its antitoxin, it is less thermostable than when free. Similar facts were adduced by Wassermann with regard to the combination between pyocyaneus toxin and its antitoxin, and are capable of a similar explanation. Marengi has also brought forward somewhat similar results with diphtheria toxin.

Lastly, Ehrlich has shown that the conditions which favour the occurrence of chemical combinations favour the union of toxin and antitoxin—*e.g.*, it is accelerated by heat, and takes place more quickly in concentrated than in dilute solutions.

This brings us to the question as to whether the combination takes place in accordance with the law of multiple proportions—a question of great difficulty, but one which has lead in its elucidation to the discovery of facts of much interest. As far as concerns the action of the hæmolysins and other toxins that can be readily tested *in vitro*, there is no doubt that this question, in its simplest form, must be answered in the affirmative. If it requires  $x$  c.c. of a given solution of toxin to dissolve exactly 1 c.c. of a 5 per cent. emulsion of red blood-corpuscles, then it will require  $2x$ ,  $3x$ ,  $4x$ , etc., c.c. to hæmolyze 2, 3, 4, etc., c.c. of the same emulsion. We assume in each case that the hæmolysin is added at once, and not in small consecutive amounts. To study the effect of the *partial* neutralization of toxin by antitoxin we will briefly outline Ehrlich's famous work on the standardization of diphtheria toxin, and the conclusions he arrived at in consequence of the results thus obtained.

We have seen that it is possible to determine with a close approach to accuracy the minimal lethal dose of diphtheria toxin for standard guinea-pigs—*i.e.*, those weighing about 250 grammes. This amount is called the toxic unit (TU), and a toxin of which  $\frac{1}{100}$  c.c. is just sufficient to kill a test guinea-pig in three or four days is considered to be normal toxin of unit strength, and is

written DTN.<sup>1</sup> A toxin of half this strength, of which  $\frac{1}{50}$  c.c. is the lethal dose, is written DTN<sup>0.5</sup>. Toxins of other potencies are numbered accordingly.

Ehrlich now proceeded to define a unit of antitoxin as the amount that would just neutralize 100 lethal doses of toxin: this is called IU (=immunizing unit). This amount may be contained in any quantity of the serum; thus, in that used for clinical work 1 c.c. contains anything between 300 and 1,000 units, or even more. For the purpose of testing toxins it is convenient to use an antitoxic serum which is much more dilute than this, and an antitoxin of unit strength is defined as one which contains 1 unit of antitoxin in 1 c.c.—*i.e.*, 1 c.c. of the antitoxin will just neutralize 1 c.c. (100 lethal doses) of standard toxin. The reaction between these amounts is written thus:

$$1 \text{ c.c. toxin (= 100 lethal doses) + 1 c.c. antitoxin} = L_0,$$

where  $L_0$  ( $L$ =limes) indicates that the mixture is a truly neutral one, and that it does not kill a susceptible animal within the time-limit, or produce any pathogenic action whatever.

Now, if, as Ehrlich believes, the affinity of toxin for antitoxin is a powerful one, similar to that of a strong acid for a strong base, it should follow that if to the 100 lethal doses of toxin we add only  $\frac{9.9}{100}$  of 1 c.c. of standard antitoxin, then  $\frac{1}{100}$  of the original amount—*i.e.*, 1 lethal dose—should remain unneutralized, and the animal should die in the same time as a similar animal which had received 1 lethal dose and no antitoxin.

As a matter of fact, this is not what occurs. We find that when we inject the mixture the animal does not die in a short time with the ordinary symptoms of diphtheritic intoxication, but develops local œdema, and possibly paralysis, which may bring about death at a remote period. The same thing happens if we add still less antitoxin to the 100 lethal doses of toxin. To take a particular case, it is not until the mixture contains less than  $\frac{7.5}{100}$  of 1 c.c. of antitoxin that the animal dies acutely in the way it does after an injection of 1 lethal dose of toxin. It seems, therefore, that the whole of the toxicity of the toxin is removed when only three-fourths of the amount of antitoxin necessary to neutralize it has been added, or that a given amount of toxin can

<sup>1</sup> DTN=diphtheria toxin normal. It is also written DTN<sup>1M</sup><sup>250</sup>=DTN one unit for a guinea-pig (*Meerschweinchen*) weighing 250 grammes.



combine with one-fourth more antitoxin than is necessary to neutralize it.

To account for this Ehrlich supposed that there are really two substances present in the broth in which diphtheria bacilli have been grown. There is the true *toxin*, which brings about local inflammatory œdema, often going on to necrosis and causing local alopecia, and causing acute death, and *toxon*, which produces only soft and transient œdema locally and subsequent paralysis. Both these substances combine with antitoxin, but the toxin has the greater affinity for that substance, and when the total neutralizing dose of antitoxin is added in successive small amounts, the whole of the toxin is neutralized first, leaving the toxon free, and this takes place when three-fourths of the whole amount of antitoxin has been added. Ehrlich represents this result in the form of a spectrum, thus :

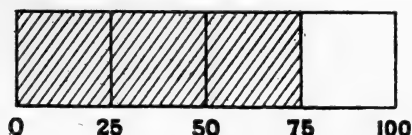


FIG. 8.—SIMPLE SPECTRUM OF TOXIN.

The rectangle represents the  $L_0$  dose of toxin—*i.e.*, in this simple case 1 c.c. of the solution. The portion with the greatest affinity for antitoxin is placed at the left hand of the "spectrum"; in this case it is represented by the toxin. On the right are the substances with the least affinity for antitoxin—in this case the toxon.

Further investigation shows that the process is not usually so simple as this. In certain samples of toxin we find that the addition of small quantities of antitoxin causes no alteration in the toxicity of the  $L_0$  dose. Thus, in a case of frequent occurrence it happens that we may add  $\frac{1}{4}$  c.c. of normal antitoxin before any loss of toxicity occurs; 1 c.c. of the normal toxin will kill 100 guinea-pigs, and 1 c.c. of the same toxin +  $\frac{1}{4}$  c.c. of normal antitoxin will still kill 100 guinea-pigs. To explain this, Ehrlich supposed that the solution contains a third substance, *prototoxoid*, which is entirely devoid of lethal activity, but which has a power of combining with antitoxin even greater than that which toxin possesses. Thus, on the addition of small amounts (up to  $\frac{1}{4}$  c.c.) of the antitoxin, this inert substance will seize on the antibody, unite

with it, and so render it incapable of neutralizing the true toxin. The spectrum of this solution will be represented thus :



FIG. 9.—SPECTRUM OF TOXIN.

In this, as in the other diagrams, the lethal portion of the mixture is shaded, the non-lethal portion left blank.

Ehrlich found on actual experiment that the constitution of the solution was even more complex than this, and had to assume the existence of yet other bodies. Thus, if the spectrum above were a true representation of the constitution of 1 c.c. of the solution, it follows that the first quarter and the last quarter of the antitoxin added were without effect, so that the middle  $\frac{1}{2}$  c.c. completely neutralized the whole of the 100 lethal doses. Now let us imagine this 1 c.c. of standard antitoxin divided into 200 equal parts, and added part by part to the 1 c.c. of standard toxin, or 100 lethal doses. Then—

The first 50 parts added will combine with *prototoxoid*, and will not affect the toxicity of the mixture ;

The next 100 parts added will neutralize 100 lethal amounts of *true toxin* ; and

The last 50 parts will combine with *toxone*.

Now if the spectrum were as simple as is shown above, and if the toxin were quite uniform in its combining capacity and its toxicity, it would follow that the first  $\frac{1}{200}$  part added after the addition of  $\frac{50}{200}$  part would just neutralize one lethal dose and leave 99 lethal doses over. Again, the addition of the amount necessary to neutralize all the prototoxoid ( $=\frac{50}{200}$  c.c.) +  $\frac{99}{200}$  c.c., which would neutralize all the prototoxoid and all the toxin except  $\frac{1}{100}$  part ( $=1$  lethal dose), and all the toxone, should leave 1 lethal dose of toxin free, and the animal should die in the limit of time for 1 lethal dose. We might represent this as follows :

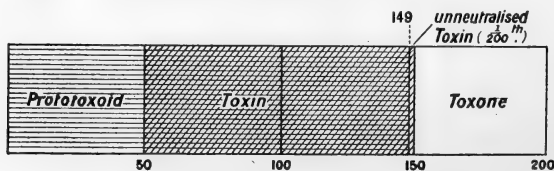


FIG. 10.

in which the oblique shading represents the toxic portions, as before, and the horizontal shading represents the amount neutralized by the addition of  $\frac{14.9}{200}$  c.c. of antitoxin; the portion with oblique but no horizontal shading represents the toxic portion which remains unneutralized: it constitutes  $\frac{1}{100}$  of the total shaded portion, and is therefore 1 lethal dose.

Such a finding may occur, but is unusual. In most cases we find that the amount of toxin left free on partial neutralization is subject to laws which are far more complex. In a case given by Madsen and described in the same way we find:

The addition of  $\frac{15.0}{200}$  parts of antitoxin left free no lethal substance—a term which we shall use for the present, instead of “toxin,” to denote the portion of the spectrum with the oblique shading. In Ehrlich’s language all had been neutralized except the toxon.

The addition of  $\frac{9.0}{200}$  left 5 units of lethal substance free; it follows that  $\frac{15.0}{200} - \frac{9.0}{200} = \frac{6.0}{200}$  had been necessary to neutralize these 5 units.

The addition of  $\frac{3.0}{200}$  left 55 lethal units free; hence, if after the addition of  $\frac{9.0}{200}$  (as above, leaving 5 lethal doses free) we add an additional  $\frac{6.0}{200}$ , the difference ( $\frac{3.0}{200}$ ) will neutralize 50 lethal doses (55 – 5).

Hence the addition of  $\frac{3.0}{200}$  will just neutralize the remaining lethal doses—*i.e.*, 45.

To account for facts like these, Ehrlich suggests that the solution contains four or five substances. The first—*i.e.*, that which has the greatest power of combining with antitoxin, is called *prototoxin*; it is lethal, and it consists of two parts—an  $\alpha$  part, which is readily changed into inert prototoxoid, and a  $\beta$  part, which is more stable, but which may, after a time, change into prototoxoid also. These two modifications have exactly the same affinity for antitoxin, so that if they were present in equal amounts, and if all the  $\alpha$  modification were changed into prototoxoid, each addition of antitoxin would go to neutralize active prototoxin and inert prototoxoid in equal amount; hence half of it would apparently be wasted.

Secondly, there is *deuterotoxin*, which also exists in an  $\alpha$  and a  $\beta$  modification, of which the  $\alpha$  part is readily transformed into deuterotoxoid, whilst the  $\beta$  modification is very stable and is the last lethal substance to disappear. The  $\alpha$  and  $\beta$  modifications have equal affinity for antitoxin, but this is less than that of the prototoxin.

Thirdly, there is *tritotoxin*, again in an  $\alpha$  and a  $\beta$  modification, with less affinity for antitoxin than deuterotoxin, and so are placed on its right in the spectrum.

It is found, further, that the proportion of  $\alpha$  modification to  $\beta$  modification in the above forms of toxin is a simple one, so that the ratio of toxoid to toxin present in any one part of the spectrum is always simple ( $\frac{1}{2}$ ,  $\frac{1}{3}$ ,  $\frac{1}{5}$ , etc.).

Fourthly, there is *toxone* (toxone) or *epitoxoid*, the characters of which we have seen.

Lastly, some researches seem to prove that there is yet another body, *epitoxonoid*, which has still less affinity for antitoxin than has toxone, and which is entirely devoid of lethal or toxic power. It will be left out of the further consideration of these bodies.

The spectrum of a toxin on this theory is recorded thus :

<i>Prototoxoid A</i>	<i>Deuterotoxoid A</i>	<i>Tritotoxoid A</i>	<i>Toxon</i>
<i>Prototoxin B</i>	<i>Deuterotoxin B</i>	<i>Tritotoxin B</i>	

FIG. 11.

The spectrum of the example given by Madsen and quoted above would be :

<i>Proto-toxin</i>	<i>Deuterotoxoid</i>	<i>Tritotoxoid</i>	<i>Toxone</i>
	<i>Deuterotoxin</i>		
30	90	150	200

FIG. 12.

Another spectrum, given by Ehrlich, is appended :

<i>Prototoxoid A</i>	<i>Deuterotoxoid A</i>	<i>Tritotoxoid A</i>	<i>Toxon</i>
<i>Prototoxoid B</i>		<i>Tritotoxin B</i>	
	<i>Deuterotoxin B</i>	<i>Tritotoxin B</i>	

FIG. 13.

We must now turn to the experimental results which have led to this idea of the change of the toxin into toxoid ; it has been

referred to several times already, but not fully discussed in order not to interrupt the main line of the argument.

$L_0$  has been defined as the amount of toxic solution which is exactly neutralized by 1 IU of antitoxin, and  $L_+$  is the amount which, when added to 1 IU of antitoxin has 1 lethal dose left unneutralized. Now if the toxic solution contained a simple substance, we should expect the two quantities to have the following relation in the simple standard toxin of which 1 c.c. contains 100 lethal doses.

$$\begin{aligned} 1 \text{ c.c. toxin} (= 100 \text{ lethal doses}) + 1 \text{ c.c. antitoxin} (= 1 \text{ IU}) &= L_0. \\ 1.01 \text{ c.c. toxin} (= 101 \text{ lethal doses}) + 1 \text{ c.c. antitoxin} (= 1 \text{ IU}) &= L_+. \\ \therefore L_+ - L_0 &= 0.01 \text{ c.c.} = 1 \text{ lethal dose.} \end{aligned}$$

This, however, is not the case. If we take a neutral mixture of toxin and antitoxin—*e.g.*, of 100 units of the former and 1 of the latter—add to it 1 lethal dose of toxin, and inject it into an animal, it will not cause death; there may be transient local œdema and late paralysis, symptoms which are indicative of the presence of free toxon. We must in general add very much more than 1 lethal dose to the neutral mixture in order to bring about a fatal result. For example, in our standard toxin it might happen that the  $L_+$  dose was about 1.35 c.c. In other words—

$$\begin{aligned} 1.00 \text{ c.c. toxin solution} + 1 \text{ unit of antitoxin} &= L_0. \\ 1.35 \text{ c.c. toxin solution} + 1 \text{ unit of antitoxin} &= L_+. \\ L_+ - L_0 &= 0.35 \text{ c.c.} \end{aligned}$$

This result can readily be explained on Ehrlich's assumption of the existence of substances of differing combining powers for antitoxin. For the sake of simplicity, we will take his earlier nomenclature, and consider the substance as made up of proto-toxoid (with a greater affinity for antitoxin than true toxin has), toxin, and epitoxoid, with little affinity, and corresponding to toxon. The spectrum of the toxin under discussion is:

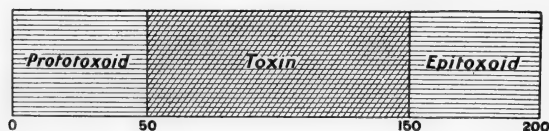


FIG. 14.

In this diagram we represent the  $L_0$  dose—*i.e.*, 1 c.c. divided into its component parts. The oblique shading represents, as

before, the acutely lethal portion, and the whole is shaded horizontally to show that it is completely neutralized by the 1 unit of antitoxin.

Now let us take 1.25 of the same solution and add to it 1 unit of antitoxin. In this extra 0.25 c.c. of toxin (a quarter of the original amount) there are 12.5 parts of prototoxoid, 25 of toxin, and 12.5 of epitoxoid. There will now be 62.5 parts of prototoxoid, 125 of toxin, and 62.5 parts of epitoxoid. The 200 parts into which we imagine the unit of antitoxin is divided will now neutralize the whole of the prototoxoid (62.5 parts), the whole of the toxin (125 parts), and 12.5 parts of toxon. There will be 50 parts of epitoxoid left free, but *no toxin*. Hence, 1.25 c.c. of the toxic solution is less than the  $L_+$  dose. The result may be represented thus :

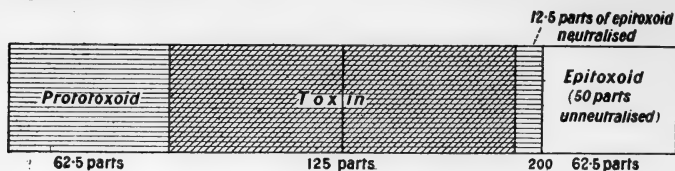


FIG. 15.

Let us now imagine a third mixture of 1.33 c.c. of the toxic solution and 1 unit of antitoxin. The 0.33 c.c. of toxin will contain 16.6 c.c. of prototoxoid, 33.3 c.c. of toxin, and 16.6 c.c. of toxon, and the total 1.33 c.c. will thus contain 66.6 c.c. of prototoxoid, 133.3 c.c. of toxin, and 66.6 c.c. of epitoxoid. The prototoxoid + toxin (= 200 parts) will just absorb the whole of the unit of antitoxin, leaving nothing but toxon free. Thus :

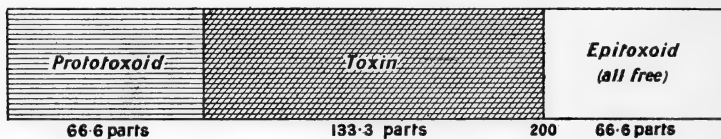


FIG. 16.

Then, if 1 extra lethal dose of toxin be added to the above mixture, it will find all the antitoxin utilized by substances with a combining affinity as great as, or greater than, its own, and will be left free. Hence, the  $L_+$  dose is just greater than 1.33 c.c.

All this follows from what has previously been said concerning

partial neutralization. If, however, we now keep this antitoxin for some time, especially if it is exposed to warmth, light, air, or certain chemical substances, we find a great change. The  $L_0$  dose is unaltered: 1 c.c. is still exactly neutralized by 1 unit of antitoxin, but we find that this amount is now much less lethal,

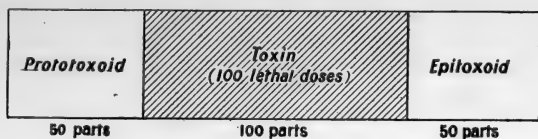


FIG. 17.

and the minimal lethal dose may have risen from 0.01 c.c. to 0.02 c.c., or higher.

If the cause for this increase in the lethal doses is investigated by the partial neutralization method described above, it will be found that the results obtained are such as will be readily explicable on the assumption that some of the molecules of toxin

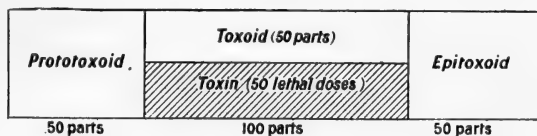


FIG. 18.

have ceased to be poisonous, but have retained their combining power unaltered; whilst the non-poisonous portions of the spectrum are unaltered. Thus, to take the simple case described above, and shown in Fig. 17, in which prototoxoid, toxin, and epitoxoid are present in the proportion of 50, 100, and 50. If we keep this, we may find the lethal dose doubled—i.e.,  $\frac{1}{50}$  c.c. instead of  $\frac{1}{100}$  c.c. But on working out the action of antitoxin, we may find that the first .25 c.c. added removes none of the toxicity, and the last .25 is equally without apparent effect. Thus the middle .5 c.c. is used up in neutralizing 50 lethal doses, and of this fraction further investigation shows that each one-fiftieth part neutralizes one lethal dose; the antitoxin, therefore, appears to have fallen off in potency; but we know that this is not the case. The explanation is that half the molecules have been changed into the non-toxic form described above. The spectrum of this altered form is shown in Fig. 18.

Thus, in a particular case Ehrlich found the minimal lethal

dose of a toxin to be 0.003, and the  $L_0$  dose 0.305 (= 100 lethal doses). Nine months later the  $L_0$  dose was still 0.305, whilst the minimal lethal dose was 0.009 c.c. (= 33.3 lethal doses only). Thus, the toxin had fallen to one-third of its former toxicity, but had retained its power of combining with antitoxin unaltered; and it is found that the two numbers usually bear some simple ratio to one another—*e.g.*,  $1 - \frac{1}{2}$  or  $1 - \frac{1}{3}$ , as in the previous examples.

We are now in a position to understand all Ehrlich's results, and especially the proof that a molecule of toxin consists of two parts—a haptophore group, which has the power of combining with antitoxin or with the protoplasm of the body cells, but which has in itself no lethal action; and a toxophore group, which has the power, when linked to a body cell by means of the haptophore group, of causing toxic symptoms, probably by a process akin to enzyme action. This toxophore group is unstable, and when it is decomposed the toxin molecule is converted into toxoid; it then retains its power of uniting with antitoxin and with the tissue cells, but is devoid of toxicity. Further, there are many varieties of toxin, which differ from one another—(1) in their avidity for antitoxin, and presumably for the tissue cells; (2) in the readiness with which they are decomposed into inert toxoid; and (3) in their toxic action, in that true toxins cause rapid death, local inflammation, necrosis, etc., but no paralysis, whilst toxons produce only transient soft oedema and subsequent paralysis. Lastly, Ehrlich supposes that toxin and antitoxin have a great affinity for one another, so that their combination resembles that of a strong acid with a strong base, and the compound toxin-antitoxin molecule when once formed does not dissociate, but remains as a stable substance. It is on this point that his views differ from the more recent ones of Arrhenius and Madsen, who regard the union as being akin to that of a weak acid and a weak base. We hope to be pardoned if, before describing their experiments and conclusions, we give a brief outline of the difference between the two reactions.

According to modern chemical theories, most substances in watery solution decompose into *ions*, atoms or groups of atoms carrying an electrical charge. Thus, a solution of hydrochloric acid contains ions of H carrying a positive charge and of Cl carrying a negative one. Different substances undergo ionization to a different degree. Thus, HCl and NaOH are ionized to a great extent, boracic acid and ammonia hydroxide but slightly.



Now in general it is only free ions which enter into chemical combination. For example, on adding HCl to NaOH the positively charged H ion combines with the negatively charged OH ion to form water, and the positively charged Na ion combines with the negatively charged Cl ion to form NaCl. The two substances HCl and NaOH are strongly dissociated, and hence the combination between the two is almost complete. This is an example of the combination of a strong acid and a strong base; where it was expressed in older phraseology, the substances have a strong affinity for one another. It is to this type that Ehrlich conceives the union of toxin and antitoxin belongs.

When two substances dissociate but slightly—*e.g.*, acetic acid and alcohol or boracic acid and ammonia—the reaction takes place in obedience to different laws (the law of “mass action” of Guldberg and Waage). When we add alcohol and acetic acid we get ethyl acetate (ester) and water; but if we take equivalent combining quantities of the two primary substances, the reaction is not complete, as it is in the case of HCl and NaOH. On the contrary, the solution will still contain free alcohol, free acetic acid, ester, and water. This is due to the fact that the process is reversible. Acetic acid and alcohol combine on the one hand to form ester and water, and ester and water combine on the other, and dissociate into free acid and free alcohol.

In the first case, in the combination of a strong acid and a strong base, the reaction is a simple one. If we take 100 combining equivalents of NaOH, and add to it 10 combining equivalents of NaOH, 10 parts of the alkali will be neutralized, and so on, the whole of the alkali being neutralized when 100 combining equivalents have been added. In the second case the reaction is more complicated, and is expressed by the law that the products of the concentrations of the substances on one side of the equation, divided by the product of those on the other, is a constant (which varies in different reactions, and can be determined by experiment). Thus in the case given:

$$\frac{\text{Concentration of acid} \times \text{concentration of alcohol}}{\text{Concentration of ester} \times \text{concentration of water}} = \text{constant.}$$

It follows that when we add a relatively small amount of acid to a given volume of alcohol, it is practically all used up to form ester, much free alcohol remaining; but each succeeding addition

of acid is divided into two parts, of which one combines with the alcohol and the other remains free. As we continue to add successive small amounts, this latter uncombined portion gets greater and greater, so that the addition of successive small volumes use up less and less of the alcohol; and it is only when there is a great excess of acid that all the alcohol is used up. Theoretically, some always remains.

The difference may be represented graphically thus :

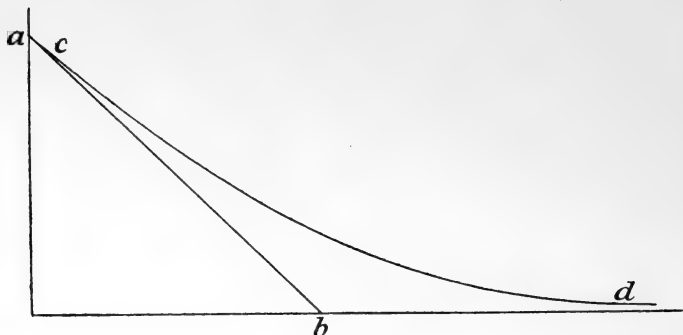


FIG. 19.

The line  $ab$  represents the neutralization of a given amount of NaOH by its equivalent of acid. It is a straight line, since each successive addition of equal amounts of acid neutralizes the same amount of alkali.

The line  $cd$  represents the reaction of alcohol and acetic acid, or, to take the example used by Madsen, the neutralization of ammonia by boracic acid. It is a hyperbolic curve. It is almost a straight line to begin with, since each small addition of boracic acid is almost all used up by the ammonia, there being enough  $\text{NH}_4$  ions to seize on all the boracic ions which are added. Farther along, however, it changes according to the rules already described, and ultimately approaches infinitely near to the base line, but never reaches it. There are always free boracic acid and free ammonia in the solution, though the amount of the latter is infinitely small when the former is in great excess.

The first suggestion that the reaction between toxin and anti-toxin might be a reversible one was due to Myers in an investigation on the hæmolytic action of snake-venom, a substance

which gives partial neutralization phenomena quite similar to those we have described as occurring with diphtheria toxin, and also declines in toxic strength, but not in combining capacity, forming toxoids. It was, however, the researches of Arrhenius and Madsen which led to the establishment of the theory on a sound basis. Madsen had previously investigated the constitution of the hæmolysin of tetanus (tetanolysin), working on Ehrlich's lines, and had found it to consist of proto-, deuterio-, and trito-toxin, and a large amount of toxone. The substance was much more convenient to work with than diphtheria toxin, since a constant emulsion of red blood-corpuscles could be used as test objects instead of guinea-pigs, and thus the experiment could be multiplied at will; and when Madsen added the antiserum in *small amounts* he found that the apparent irregularities due to the presence of these various forms of toxin disappeared, and the curve of neutralization is represented by a curve similar to that given above, as illustrating the reactions between boracic acid and ammonia. Thus:

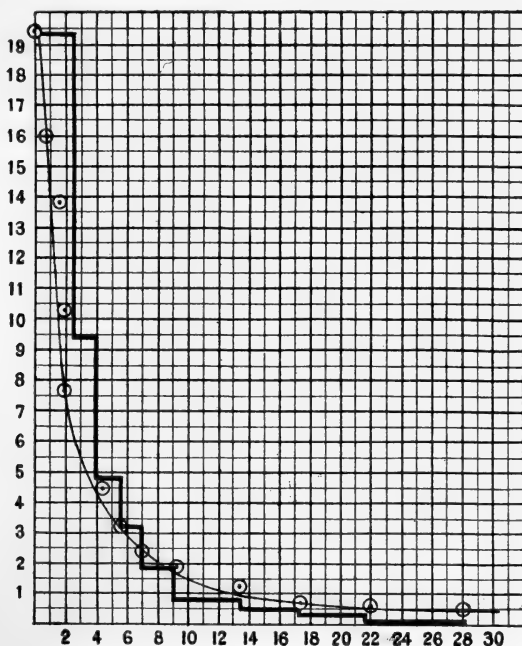


FIG. 20.—CURVE OF NEUTRALIZATION OF TETANOLYSIN (MADSEN).

The heavy line represents the curve of neutralization on Ehrlich's principles, and apparently shows the presence of a large number of varieties of toxin with different combining capacities. When, however, the neutralization was carried out by the addition of very small quantities of the antitoxin at a time, the curve became a hyperbola of the character seen in the diagram. Further, having found the value of  $k$  by determining by experiment the amount of toxin left free after the addition of a certain amount of antitoxin to a certain amount of the toxic solution in two different instances, and hence, by use of the formula :

$$\frac{\text{Free toxin}}{\text{vol.}} \times \frac{\text{free antitoxin}}{\text{vol.}} = k \frac{(\text{toxin} \times \text{antitoxin})}{\text{vol.}},$$

the amount of free toxin after any addition of antitoxin could be calculated theoretically. It could then be determined by experiment, and the results compared. In one case given by Arrhenius and Madsen this was done, with the following result :

Amount of Antitoxin added.	Amount of Toxin (observed).	Amount of Toxin (calculated).
0.05	3.67	3.67
0.1	3.13	2.95
0.15	2.32	2.29
0.2	1.62	1.72
0.3	0.97	1.03
0.4	0.63	0.62
0.5	0.45	0.46
0.7	0.27	0.28
1.0	0.18	0.18
1.3	0.12	0.13
1.6	0.09	0.11
2.0	0.08	0.09

The correspondence is certainly very close.

The case of diphtheria toxin was next investigated, and in the figure which follows the "stair-step" curve, showing Ehrlich's conception of its constitution, shows the presence of proto-, deutro-, and trito-toxins, and of toxon. The curved line is that calculated after the constant of dissociation had been determined by experiment.

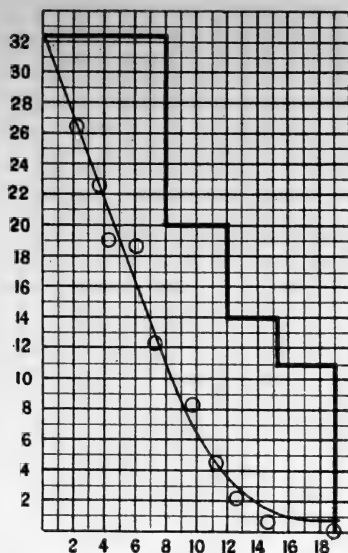


FIG. 21.—CURVE OF NEUTRALIZATION OF DIPHTHERIA TOXIN (MADSEN).

This table shows the difference between the observed and calculated results :

Antitoxin.	Free Toxin (observed).	Free Toxin (calculated).
0.1	75.1	75.1
0.15	62.6	62.7
0.2	47.6	50.6
0.25	45.8	38.6
0.3	25.9	27.3
0.35	17.3	17.5
0.4	9.6	9.6
0.45	5.3	6.0
0.5	3.1	4.1
0.6	1.6	2.6

The conclusion to which Arrhenius and Madsen came was that toxin and antitoxin react like a weak acid and a weak base ; that the reaction is a reversible one ; and hence that the combination of toxin and antitoxin is an unstable one, dissociating into free toxin and free antitoxin.

On this theory many facts which were formerly very difficult to explain become quite simple. Take, for instance, the exact point of neutralization of toxin by antitoxin as seen in the determination of the  $L_0$  dose: this has always been a matter of great difficulty—a difficulty formerly explained by assuming the last substances to be neutralized are the toxons, which have a very feeble and indefinite pathogenic action. On the physical chemistry theory the difficulty disappears, because there *is* no point of exact neutralization. In spite of the presence of an excess of antitoxin, some dissociation of the toxin-antitoxin molecule will always occur, and the mixture will always contain free toxin, though in very small amount. Further, an exactly neutralized mixture containing a few lethal doses of toxin is, of course, without action, whereas a large bulk of the same mixture may be toxic; this also is readily explicable. Then there are some old experiments of Buchner's, which showed that a mixture of tetanus toxin and antitoxin which was neutral to mice would produce tetanus in guinea-pigs, and several others (Roux's and Roux and Vaillard's) of similar nature. The explanation of these is also easy.

The most interesting explanation of a previously known phenomenon which Madsen offers in the light of his new theory is that of the immunization of animals by means of a neutral mixture of toxin and antitoxin. If we follow Ehrlich, and believe that the compound is a stable one, this is very difficult to explain. Madsen's solution is that the mixture contains free toxin, to which the immunizing property is due.

Again, we can also explain the death of animals from specific intoxication when it occurs in spite of the presence of free antitoxin in the blood in exactly the same way. It is true that the free antitoxin would tend to inhibit dissociation; but, on the other hand, the hypersensitiveness of the tissues which occurs at the early stages of the process of immunization—and it must be remembered that it is only in these stages that death from intoxication takes place—would render the cells more susceptible to minute amounts of toxin. The explanation may not be a perfect one, but it appears to be the best forthcoming.

The importance of the whole question from the point of view of immunity rests on this question of the dissociability of the combination, for it is obvious that if this is the case, our views of the action of antitoxin in the animal body will be very different from those we shall hold if we regard the toxin-antitoxin molecule

as an inert one of no further interest; and this theory of dissociation is open to the objection urged by Nernst, and probably felt by most bacteriologists on the first enunciation of the views of Arrhenius and Madsen. Thus, if the combination of toxin and antitoxin undergoes dissociation in the living animal and the toxin is set free, it will immediately combine with the susceptible cells. The equilibrium will now be disturbed and more toxin-antitoxin molecules will be dissociated, more toxin set free, and more cells poisoned; and this process will go on until all the toxin has been passed on to the cells and the antitoxin left free. Now this dissociation takes place quickly, so that on this theory it would seem that the antitoxin would only interpose a very temporary barrier between the cells and the toxin, the lethal action of which would be delayed, but in no way inhibited. We will return to this question of dissociation shortly, and meanwhile state briefly Ehrlich's objections to the physical-chemical theory. In the first place, he points out that if we make a mixture of two alkaloids, of which one is hæmolytic and the other not, and neutralize them by the addition of a strong acid, the result may be represented by a hyperbolic curve; this is put forward as a parallel experiment to the neutralization by antitoxin of a substance containing active toxin and inert toxoid. Secondly, some of the curves given by Madsen and Arrhenius do not correspond very closely with the observed results, and this is especially the case at their commencement and termination. At the commencement of the curve it is found in many cases that the addition of small amounts of antitoxin does not influence the toxicity; this is readily explained on the supposition of the existence of prototoxoid, but hardly on any other hypothesis. The most interesting point, however, is the behaviour of the curves at the termination—*i.e.*, in what Ehrlich would call the region of the toxons; here Arrhenius and Madsen usually found figures which were lower than the calculated results. Now Ehrlich holds that traces of toxin do not lead to paralysis, and if the effect of a nearly neutral mixture of toxin and antitoxin were due to dissociation we should expect no paralysis to occur, the toxic action being due to toxin, and not, as Ehrlich thinks, to toxon. Madsen and Arrhenius suggest that the action of this trace of antitoxin may be modified by the presence of antitoxin in excess. Further, Madsen and Dreyer claim to have found a diphtheria poison, of which small quantities would cause paralysis without the addition of antitoxin, so that the question of toxon

would not come in. And they also showed that certain mixtures of toxin and antitoxin might act fatally on rabbits in a few days and cause only paralysis in guinea-pigs, but that if a little more antitoxin were added it would act as a toxon on rabbits and be inert to guinea-pigs. To explain this, Ehrlich had to add yet another body to his list of components of the diphtheria poison, and to the proto-, deutero-, trito-toxin, and toxon he added toxonoid, which is inert for guinea-pigs and produces paralysis in the rabbit. The subject will not be followed farther, but enough has been said to show its extreme difficulty.

To revert to the question of dissociation, Madsen and Walbum have brought forward some definite evidence of its existence, of which the more important are the following. They neutralized ricin with antiricin, and to the neutral mixture added some red blood-corpuscles. After allowing the mixture to stand for some time, the latter were centrifugalized off, when it was found that they had become charged with ricin and the fluid contained free antiricin.

Secondly, they made use of the fact that diphtheria toxin is a substance of comparatively small molecule, and can diffuse through gelatin, whereas its antitoxin does so very slowly. They prepared a neutral mixture of the two, and placed it on the surface of a column of gelatin, and after some forty days' contact examined slices at different depths, and found free toxin at a certain distance from the surface. This they explained by assuming that it had dissociated from the antitoxin, and diffused downwards into the gelatin. It was pointed out, however, that if the mixture had been allowed to stand for some time the phenomenon did not occur; it would seem, therefore, that the combination takes place slowly, but, once formed, does not dissociate.

There is, however, other and independent evidence in favour of the theory that dissociation of a primary substance and its antibody does occur. Thus Muir and Morgenroth showed independently that if red corpuscles are treated with as much amboceptor as they will take up, and then mixed with normal corpuscles, some of the amboceptor will pass to the latter, and on the addition of a suitable complement-containing serum the whole may be dissolved. (In this experiment the red corpuscles correspond to the toxin, whilst the amboceptor is the antibody and corresponds to the antitoxin.)

Bordet's explanation of the phenomena is quite different. Both



Ehrlich on the one hand and Arrhenius and Madsen on the other agree that the combination of toxin and antitoxin is a chemical union, and takes place in obedience to the laws of multiple proportions: a single molecule of the one substance always combines with the same number of the other on complete neutralization. Bordet denies this, and compares the phenomenon with the absorption of a stain by a colourable substance. His theory is that a molecule of toxin requires many molecules of antitoxin for its complete neutralization, and that it can be partially neutralized or attenuated by a smaller number. Thus he holds that when a small amount of antitoxin is added to a large amount of toxin, there is not a mixture of free toxin and of molecules of toxin-antitoxin (as would occur if either of the theories already discussed was true), but a uniform solution of toxin of diminished potency. Bordet shows that many of the experimental results of other observers are explicable on his theory, and gives some new facts in support thereof. For example, the amount of an emulsion of red blood-corpuscles which can be hæmolyzed by the addition of a given quantity of hæmolytic serum can be readily determined. We will suppose that 1 c.c. of the serum just dissolves all the corpuscles in 5 c.c. of the emulsion, and no more. We might suppose that all the red corpuscles have all their combining valencies exactly neutralized, so that we may regard them as an exactly neutralized toxin-antitoxin mixture. This, however, is not the case, for if we add the hæmolytic serum in small amounts, say 0.05 c.c. at a time, we shall find that only a small proportion, perhaps 2 c.c., of the same emulsion can be completely hæmolyzed. Thus each corpuscle takes up more hæmolysin in the second case than in the first.

The objection may be raised that a red corpuscle is very different from a molecule of toxin. It can undoubtedly combine with a vast number of molecules of its antibody (hæmolysin), but it is quite easy to understand how complete hæmolysis may take place if only a certain number of its combining valencies are occupied by antibody. But the molecule of toxin is, as we have already seen reason to believe, much smaller than the molecule of antitoxin; and although this fact does not in itself render it impossible that the toxin is of higher valency than the latter, it certainly renders it unlikely that it is so much higher in this respect that there can be an indefinite number of stages between free toxin and a fully neutralized one. Bordet has, however,

brought forward evidence in favour of his view to which this objection can hardly apply. It will not be discussed here, as it involves certain questions concerning serum hæmolysins which have not yet been discussed.

Bordet's theory supplies an explanation, though hardly an adequate one, of the negative phase. We have already seen that when an animal is producing antitoxin an injection of toxin will cause a fall in the antitoxic value of the serum far greater than can be accounted for by the neutralization effected by the toxin injected: it may be 2,000 times as great, or more. If we assume that the combination does not take place in obedience to the laws of multiple proportions, we can imagine that it may go on very differently in the body, and that in that situation a small amount of toxin may neutralize a large amount of antitoxin. But it is very difficult to believe that this is the true explanation, for it would involve an increase of the neutralizing power of the toxin to 2,000 times that which it has outside the body—an increase which certainly seems improbable. Further, we have been asked to imagine a molecule of antitoxin spreading itself out and weakening many molecules of toxin, and we are now asked to imagine a molecule of toxin dividing itself amongst 2,000 molecules of toxin, and completely neutralizing them.

The last conception which we have to consider is that of Biltz, which approaches very closely to Bordet's. Starting with the idea that toxins and antitoxins are both colloids, he attempts to show that their union is analogous to adsorption rather than to an ordinary chemical union.

Adsorption is a process akin to solution, and does not necessarily involve a chemical union between the two substances which take part in it. It may take place between colloids and colloids, or between colloids and crystalloids, and in other ways. The two substances taking part in the process are found to be electrically positive and negative (as shown by their moving to the cathode or anode in an electric field); thus a colloidal solution of silicic acid (which is negative) is precipitated slowly by  $K_2SO_4$ , which has a feeble positive charge; more rapidly by  $CuSO_4$ , which has a stronger one; and immediately by  $Al_2(SO_4)_3$ , which has a stronger one still.

Biltz and his collaborators started with the idea that toxins and antitoxins are both colloids. They attempted to find the electrical nature of tetanus toxin by electrolysis, but found that it was

destroyed; the destruction occurred, however, sooner at the cathode than at the anode, suggesting that it was a negative colloid. They found it to be precipitated by positive colloids, such as colloidal hydrated oxide of iron or chromium, though this action occurred only *in vitro*, not *in vivo*. Tetanus antitoxin, however, did not lose its activity when exposed to mineral colloids.

Further, there is an approach to the phenomenon of specificity in the adsorption of one colloid by another, since certain colloids are only precipitated by certain other colloids; the specificity, however, is by no means so exact as in the case of the toxins, etc., and their antibodies.

A further analogy arises in the explanation of the difference between the  $L_0$  and  $L_+$  dose, which is paralleled by the relationship between colloidal ferric hydroxide and arsenious acid. When these two substances are mixed adsorption and precipitation take place; hence the use of the former substance as an antidote for the latter. Now it is found that if a solution of arsenious acid is just rendered toxic by the addition of ferric hydroxide, very much more than one lethal dose of arsenic must be added to make the mixture toxic again.

The further investigation into the evidence which they have adduced would lead us too far into the study of the agglutinins to be undertaken here. It will be dealt with subsequently (see Chapter XII.).

It seems, on the whole, that no theory is absolutely sufficient to explain all the phenomena, and that as soon after each new one is adduced the supporters of the older ones bring forward evidence which renders it untenable. The probability is, at the time of writing, that Ehrlich's views are generally held, and are open to the fewest objections. They are complicated, it is true, and have had to undergo constant modifications as new facts have arisen; but the facts themselves are complicated. Yet it must be confessed that there are some grave objections to its acceptance in its present form, and it may become yet more involved before it can be fully accepted as a complete explanation. Thus the analogy with other bodies, and the phenomena of the death from intoxication of animals with antitoxin in their blood, seem to point strongly to the theory that the toxin-antitoxin molecule dissociates strongly both *in vivo* and *in vitro*. Yet this is not compatible with Ehrlich's views.

## CHAPTER V

### THE ORIGIN OF ANTITOXIN—THE SIDE-CHAIN THEORY

THE theory that antitoxins are derived from their appropriate toxins deserves a short consideration, since it is so inherently probable. When we consider the large numbers of toxins which give rise to their antitoxins when injected into animals, and see that each antitoxin has a direct action on its own toxin, but none or practically none on others, the most likely explanation of the phenomenon is that the animal tissues have the power of splitting the toxin into two parts, or of otherwise modifying it, and that this modified toxin can combine with unaltered toxin and form antitoxin. Some experimental evidence is forthcoming to support this view. Thus it is found that diphtheria toxin which has been submitted to the action of an electric current loses its toxicity, but retains that of producing immunity on injection. Some thought that this was due to the direct transformation of the toxin into "artificial antitoxin," and it was hoped that a similar change might be brought about in the human body in disease by the use of electricity. The explanation of the phenomenon is very simple. The electrolysis of the water in which the toxin is dissolved gives rise to oxidizing substances which transform the toxins into toxoids, the immunizing virtues of which we have already seen. There is no method by which antitoxin can be prepared other than by the injection of toxins into suitable animals.

That antitoxin is not a direct transformation product of toxin appears probable from the following considerations, none of which perhaps is conclusive, but which together make up a body of evidence of some importance :

1. The injection of a certain amount of toxin will give rise, under suitable circumstances, to the formation of much more

antitoxin than it can neutralize. Thus Knorr showed that a single unit of toxin might call forth 100,000 units of antitoxin. This can scarcely be accounted for by supposing the transformation of the one substance into the other.

2. It frequently happens that antitoxin occurs, sometimes in considerable amounts, in the blood of animals that have not received injections of toxin, and have not suffered, as far as known, from the disease in question—in other words, of “normal” animals. Thus horses frequently have traces of diphtheria antitoxin in the blood, as much as 4 units per c.c. having been observed. Metchnikoff suggests that this may be due to the action of “pseudo-diphtheria” bacilli, which are so common. But no one has been able as yet to produce diphtheria toxin or symptoms of diphtheria by means of these bacilli, and it is not usual to regard them as having any connection, other than a superficial morphological resemblance, with the true diphtheria bacillus.

If we may bring other antibodies into the argument the case against Metchnikoff's supposition is enormously strengthened. The blood of many animals contains agglutinins and hæmolysins—often in large amounts, often under conditions in which we may almost exclude the possibility of a preceding injection. Thus the blood of a normal horse will almost invariably clump typhoid bacilli, *B. pyocyaneus*, the cholera vibrio, and many other organisms.

3. An animal which has received an injection of antitoxin rapidly eliminates it from the blood, whereas the antitoxin which is formed in the body remains a much longer time. This argument does not carry very much weight, since, as Metchnikoff points out, the toxin might remain for long periods occluded in the cells, and only undergo a gradual transformation into antitoxin.

4. Roux and Vaillard showed that the whole of an animal's blood might be removed by repeated venesections, and that the newly regenerated blood might contain almost as much antitoxin as was present before the hæmorrhage, thus suggesting a new formation. But the objection urged under the last heading is of weight here also, and if we consider it probable that toxin may remain latent in the tissues for long periods without causing symptoms, we shall exclude this piece of evidence from the argument, as well as the observations of Salomonsen and Madsen,

that the production of antitoxin may be increased by an injection of pilocarpin.

If we reject this explanation of the phenomenon, and regard the production of antitoxin as being analogous to the process of internal secretion, we must regard it as being due to the action of the living cells when they are stimulated by the toxin. There is at the present time but one theory which suggests how this may be brought about: this is Ehrlich's celebrated *side-chain theory*, which has played so important a part in the modern study of problems concerning infection and immunity, and which, though highly hypothetical, deserves a close and careful study.

Ehrlich points out that a cell has two functions. The first is the physiological function, which it enacts in the body—secretory in the case of a gland cell, conductive in the case of a nerve fibre, etc.; and the second is the function of nutrition, necessary to supply the waste which is constantly taking place. We must suppose a similar constitution for each of the complex molecules of living protoplasm which build up the cell; in each molecule there is a portion which discharges the specific function of the cell, and this portion has to be nourished. The functional portion is the more important, and is called by Ehrlich the active centre (*Leistungskern*). In our diagrams we shall represent it as forming the central portion of the giant molecule, but it is hardly necessary to caution the reader that this diagrammatic representation has no necessary morphological basis. It is just as permissible to regard this portion as diffused through the nutritive part of the molecule as long as the difference in constitution and action of the two are borne in mind.

The second portion, that concerned in nutrition, is more important in relation to immunity. We may regard it as having two functions—it “seizes” suitable molecules of food substances from the blood or lymph in which it is bathed, and it alters them in such a way as to build them up into living material which can take its place in the molecule of protoplasm, so as to replace that which has been used in the life of the cell.

The function of “seizing” molecules of food from the surrounding tissues implies a selective faculty, for we cannot imagine that all the molecules which circulate in the blood and lymph are suitable for all cells at all times. This apparently intelligent selection of suitable material is, of course, at bottom a chemical process: the food molecule becomes attached to some portion of

the cell for which it has a chemical affinity. Now Ehrlich supposes that this affinity for food molecules is situated in certain portions of the molecule of protoplasm—in certain groups of atoms which he calls *side-chains*, *receptors*, or *haptines*. [The name “side-chain” was, perhaps, badly chosen. It denotes a possible analogy with complex organic bodies—*e.g.*, of the aromatic group—which are composed of a central portion (such as the benzene ring) and side-chains, on which many of their reactions depend. The comparison is not a very close one, and all that is necessary is to regard the molecule of protoplasm as possessing numerous groups of atoms, each of which has an affinity for one of the bodies circulating in the body fluids, and necessary for the life of the molecule in question.]

On this theory the nutrition of the molecule takes place as follows: A molecule of suitable food substance in the fluid surrounding the cell is brought into contact with one of the receptors for which it has a chemical affinity, and the two unite. This is the first step in the process. The food is “anchored” to the cell by means of a receptor, for which it has a specific combining affinity, or which, to use Ehrlich’s analogy, fits it like a key fits a lock. The second stage involves a process which we may compare to digestion, by which the food molecule is altered in some profound manner, and absorbed, in whole or in part, into the molecule of protoplasm.

Let us apply this theory to the process by which a cell is poisoned. We will imagine for a moment that the molecule of toxin contains a group of atoms which will unite specifically with the side-chain of one of the body cells. We have already shown that this molecule of toxin contains a haptophore group of molecules, in virtue of which it can combine with antitoxin, and a toxophore group, on which its toxic action depends. We can now go farther, and say that the first stage of the intoxication of a cell by means of a true toxin consists in the union of the haptophore group of atoms in the toxin to a receptor of a molecule of protoplasm, this receptor being one which “fits it like a key fits a lock.” Each molecule of protoplasm has innumerable receptors, of which only a certain number are suitable for this toxin. This is the first step. The toxin molecule is now “anchored” to the living cell, and in the second stage the toxophore radicle of the toxin comes into play. We may regard this toxophore group as exerting an enzyme-like action on the protoplasm through the

haptophore group and the receptor, by which the two are united. Thus :

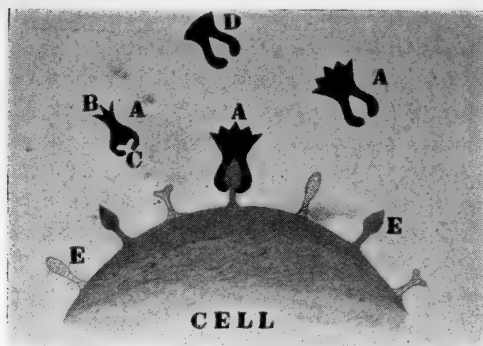


FIG. 22.—CELL MOLECULE WITH RECEPTORS (E, E).

A, A, Molecules of toxin (B=toxophore, C=haptophore), D=a molecule of toxoid.

The result of this is that the protoplasm is poisoned. If only a few of its receptors are united to toxin molecules the result may be but slight, whereas if more are occupied the functioning centre of the molecule will be affected, and marked symptoms will arise, and if still more molecules of toxin are linked up the molecule of protoplasm may be killed.

The essential point in this process is that it is exactly analogous to natural nutrition, except that in the latter case the receptor unites with a molecule which is of use to the cell, whereas in the former it unites with one which simply resembles the food molecule in having a haptophore group with similar chemical affinities. Thus, *intoxication with bacterial toxins is essentially a process of nutrition*, but is perverted in its later stages by the nature of the toxophore group of the toxin.

Now consider the case in which a molecule of protoplasm is attacked by a number of molecules of toxin which is not large enough to kill. A considerable number of receptors will be taken up by toxin, and we must consider these receptors as being thereby rendered useless. The protoplasm, however, has need of these receptors, and the need may probably be more pressing since it is poisoned, and metabolic changes may take place more rapidly, and there is thus greater need for renewed nutriment. Fresh receptors must therefore be formed, and Ehrlich compares their regeneration to the budding forth of fresh tentacles in the hydra, to replace



those which have been lost. Now suppose a fresh dose of toxin reaches the cell, and that these new receptors are in their turn taken up by toxin, but yet not in numbers sufficient to kill the cell. The same processes will occur: the receptors will be rendered useless, and a fresh crop of the same nature will be produced.

Now in accordance with the well-known laws of habit, in virtue of which a part of the body can gradually be trained to perform a function which is difficult at first, but which becomes more easy by usage, the molecule of protoplasm gradually acquires the faculty of producing these receptors more and more easily, in obedience to nicely graduated doses of toxin. If these are repeated in proper amount and at suitable intervals the cell may be trained, so to speak, to produce these receptors, and it may

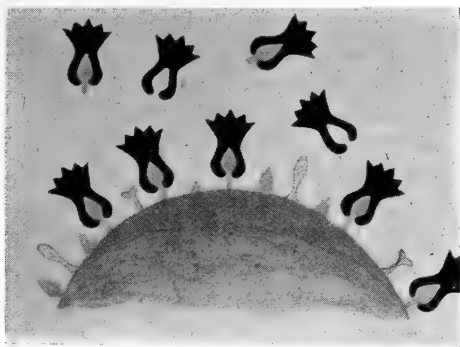


FIG. 23.

ultimately come to do so in excess and far beyond its physiological requirements. This also is analogous with known biological facts, such as the production of callus in a fractured bone in excess of the amount necessary for repair. We shall revert to this point later. Now we have to imagine that these receptors may be formed in excess so great that they cannot all remain attached to the cell; some of them are pushed off, so to speak, by the younger ones, which are growing up to take their place. If this occurs, these receptors will pass off freely into the blood. As we have seen, they are so constituted that they will unite specifically with the particular toxin that was injected. *These cast-off receptors constitute antitoxin*, and the formation of that substance is simply due to the pathological separation of the groups of atoms, in virtue of which the toxin molecule is linked up to the living

protoplasm. The whole process is explained as one of perverted nutrition, and (once admitting Ehrlich's theory of the constitution of the molecule of protoplasm and of the way it is nourished) without introducing any but well-recognized biological phenomena. The theory is fascinating in its simplicity, and although there are a few difficulties in the way of its full acceptance as a complete explanation of the facts, it certainly accounts for them far better, on the whole, than any other theory will do—if, indeed, an alternative one has been put forth. And it has the great merit in a theory that its application has led to the discovery of new facts. '

A few remarks are necessary in connection with the question



FIG. 24.

of the mechanism in which the over-production of side-chains is produced. Weigert's hypothesis with regard to the nature of hyperplasia as the result of injury or irritation is now well known, and a brief outline will suffice. He imagines that the maintenance of the normal structure and physiological function of a tissue depends upon a condition of equilibrium brought about by a series of mutual restraints exerted by each cell on its neighbours. In the same way, the structure of a single cell, or perhaps of a single compound molecule of protoplasm, depends on a similar equilibrium existing between minute living units. If one of these components is killed or injured, the restraint which it exerts on the surrounding units is removed, and unrestrained growth can

take place; and Weigert points out that this always goes on to excess, more new material being formed than is necessary to replace the amount lost. The application of this theory to the mode of new formation of receptors is sufficiently obvious.

We will now consider certain phenomena in the light of the side-chain theory.

The occurrence of antibodies in the blood of normal animals is susceptible of a ready explanation. The receptors are, in general, united to the cell, but it is easily conceivable that a few may be desquamated accidentally and escape into the blood. It must be remembered that the actual amount (weight or bulk) of these antibodies in the normal blood is probably always infinitesimal, although the effects of this small amount may be striking. Thus the agglutination of typhoid bacilli at a dilution of 1 to 100 (which often occurs with the blood of normal horses) indicates the presence of a very small amount of specific antibody, as is apparent from the fact that the blood may be made to clump at 1 to 1,000,000 without being altered chemically in any way that can be detected. If an exceedingly minute proportion of all the receptors are shed into the blood, it will probably account for all the antibodies found in that situation under normal conditions.

The fact that toxoids produce antitoxin<sup>1</sup> is also explicable. We must regard the receptor which is occupied with toxoid as being thereby rendered useless for the protoplasm, although the latter is not poisoned as a result of the union.

Next arises a most important question, and one which is apparently satisfactorily answered on the side-chain theory—the fact that certain substances (bacterial toxins in particular) give rise to the production of antibodies, whilst others, such as the alkaloids, glucosides, mineral poisons, poisons of simple constitution, such as alcohol, etc., do not. (This may be taken as fairly proved, certain researches which go to prove the existence of antibodies to morphine, alcohol, etc., being inconclusive.)

The explanation is founded on differences in the way the various substances form combinations with the protoplasm. This latter has, as an integral part of its constitution, certain receptors which have a specific combined affinity for proteids, and we must imagine the union of proteids, whatever be their nature, as taking place by a direct union with these receptors. Other substances,

<sup>1</sup> According to Ehrlich. The fact is not universally admitted, though all agree that they will produce immunity.

however, need not, and in all probability do not, unite with the protoplasm in this way. Ehrlich, basing his theories on the fact that certain alkaloids and other substances can be extracted from their combinations with organic matter by simple means, has suggested that the union of non-proteid poisons with protoplasm is less firm than in the other case. This seems doubtful, for nothing could be much firmer than the combination of nitrate of silver or similar bodies with the tissues. And we know as a matter of fact that the toxin-protoplasm compound is not necessarily a stable one. Emulsions of tissues will abstract tetanus toxin, and retain it on washing, but give it up again in a free state when allowed to stand for some time in contact with normal saline solution. However this may be—and the point is not of importance—we may readily admit with Ehrlich that it is highly probable that proteids unite with protoplasm in a manner fundamentally different from alkaloids, etc. The former process follows on physiological lines, whilst the latter is a pathological process entirely, and one which has no counterpart in normal nutrition. The former may be compared to the insertion of the key in the lock, the latter to the violent smashing of the lock. If this is the case, it is easy to see that “chemical” poisons cannot be expected to give rise to the production of antibodies. It is true that if a cell is already secreting antibodies, we might expect that a substance which stimulates the general metabolism of the cell and increases the rapidity of the vital processes might temporarily increase this production, and we have seen that this is the case with pilocarpin, which stimulates the production of diphtheria antitoxin in an immunized horse. This is an entirely different process, and one that is easily explicable on the side-chain theory.

It is necessary, therefore, to determine whether all the substances which give rise to the production of antibodies on injection are proteids. We must admit at the outset that in many cases this cannot be proved; the chemical constitution of toxins, enzymes, and many other antigens is as yet quite unknown. But in the cases in which the chemical composition of the primary substance is ascertained we find it to be invariably of proteid nature; thus, solutions of any of the coagulable proteids will give rise to the production of precipitins; the proteid substances present in the body of bacteria give rise to the production of agglutinins, which are themselves of a proteid nature, and will, on injection into suitable animals, give rise to anti-agglutinins; the proteid con-

stituents of the red blood-corpuscles, the substances forming their stroma, call forth the production of hæmolysins; and a great many other cases might be quoted. It may be taken as definitely established that wherever the nature of the antigen is known and falls into one of the known groups, that antigen is a proteid.

We may almost go a step farther, and say that all proteids, of whatever nature, when injected into suitable animals, will give rise to antibodies; in fact, some writers actually enunciate this as a law. There are, however, a few exceptions, which are gradually diminishing in face of further researches, and it is highly probable that time will show that the law is valid to the fullest extent.

Reverting now to the question of the nature of the bodies of unknown constitution which form antibodies—*i.e.*, the toxins, enzymes, etc.—we may ask whether these are not in reality proteids, in spite of their failure to give the proteid reactions as usually accepted by chemists. The question is largely one of nomenclature. Accepting the definitions usually given in physiological textbooks, and based on a study of ordinary proteids, such as egg-albumin, etc., we may admit that they are not; but are these definitions valid? Might we not define them with greater accuracy as substances from which animal organisms can obtain food material containing combined ("organic") nitrogen? In this case we should substitute for coarse chemical experiments *in vitro* the more delicate reactions of the living animal body; any nitrogenous substance which is recognized by living protoplasm as being a suitable pabulum for it would be defined as a proteid. If this is the case, we are thrown back at once on Ehrlich's theory, and can define the proteids as substances which unite in a specific manner, haptophore to receptor, with the living molecule of protoplasm. If we do this, we may admit that the toxins and enzymes, whatever their chemical reactions, and whether they are or are not food substances for the cells, combine with protoplasm in the manner which is characteristic of proteids, and are to be regarded as proteids when looked at from this point of view. And we must not forget that many of the substances which we regard as toxins are, as a matter of fact, of value in nutrition to the animal which forms them, and only act as poisons in strange species. Thus, the ichthyotoxin of eel serum must nourish the cells of the eel, but is a powerful toxin to almost all other species. Whether any animal can utilize tetanus toxin,

diphtheria toxin, etc., as cell pabulum is doubtful, but there are some experiments which go to show that this is the case. Thus, the rat is relatively immune to diphtheria toxin, and shows no symptoms after the injection of an amount which is lethal for many rabbits, and this immunity is not due to the presence of antitoxin. This being so, it seems clear that the diphtheria toxin disappears from the blood of the rat in virtue of forming a combination with the cells of the latter without injuring them, and we may fairly assume that it is of value in nutrition, although, of course, this assumption is not open to direct proof. In a similar way tetanus toxin disappears rapidly from the blood of scorpions, no antibody being formed.

Thus it appears to be a logical sequel of the acceptance of Ehrlich's theory that we may define proteids as substances which, when injected into suitable animals, give rise to the production of antibodies. We have now to glance for a moment at the question of the suitability of different animals for the production of antibodies.

Researches with precipitins show that the proteid substances which are present in the serum of any species of animal are different from those which occur in any other. We must regard the molecule of proteid of any type (say of a globulin) as being of great complexity, and capable of many slight modifications, which are inappreciable to gross chemical tests, but which are perfectly obvious to the living animal cells. Human globulin differs from horse globulin, and this, again, from sheep globulin. Further, some facts go to show that differences exist between the proteids of animals of the same species, and that the proteids in the serum of one man or horse are not exactly the same as those in another man or horse. These differences come in as a result of the last step in the process of digestion; the food materials are broken down into simpler bodies in the alimentary canal, and built up again in their passage through the epithelial cells which line that structure, and in this process receive certain distinctive features peculiar to the species or to the animal in question. As a result, the body fluids of any animal contain proteids which have been deliberately adapted for the nutrition of the cells of that animal, but which may be quite useless or even toxic for those of another species. The prime requisite of their suitability for nutritive purposes is that the proteid molecules should possess haptophores which "fit" the receptors of the cell molecules; the second,

equally important, is that the proteid molecules shall be "digestible" by the cell. If the first requisite fails, the proteid molecule fails to unite with the cells; it may remain for long periods in the blood (as is the case with tetanus toxin in the blood of *Oryctes*, where it remains months after injection), or may probably be eliminated with the excretions, or be otherwise dealt with. In this case no antibody can be formed.

When the injected proteid molecule finds suitable receptors and unites with them, but when the resulting compound is not suitable for the nutrition of the cell, and cannot be "digested" by it, the case is different. The receptors which are occupied by the molecules of proteid are useless for the cell, and the conditions which we have discussed in dealing with the action of toxins are reproduced, although there may be no toxic action; the receptors are useless and have to be regenerated, and under suitable circumstances may be produced in excess and form antibodies. The criteria of the suitability of any animal for the production of an antibody to a given proteid are therefore three in number:

1. This proteid must not exist naturally in the blood of the animal.
2. It must possess haptophores which "fit" the receptors of the animal cells.
3. The compound thus formed must be "indigestible" by the cell and useless for its nutrition.

These are the theoretical criteria, which are derived from a study of the side-chain theory, and, on the whole, experimental research agrees with them, and thus corroborates the theory to an extraordinary extent. For example, we need scarcely refer to criterion 1: we know that precipitins do not occur normally in the blood of any animal to the proteids which circulate in that fluid; it is only the injection of a foreign proteid which causes their production. Further, the more closely allied are two animal species, the less the production of precipitin when serum from the one is injected into the other. Thus, in order to prepare a potent antihuman serum, the blood of a man must be injected into a rabbit, fowl, etc., not into a monkey. If it is true, as appears to be the case, that the serum of an animal causes the production of precipitins when injected into another of the same species, this does not affect the argument, for these precipitins only occur in minute amount, and simply show that the fluids and tissues of different animals of the same species are not in reality identical.

There is nothing surprising in that when we consider how different persons vary in constitution and susceptibility. Again, it is not uncommon to find auto-agglutinins in human blood—*i.e.*, substances which agglutinate human red corpuscles. When this is the case, it is found that these agglutinins have no action on the red corpuscles of the persons from whom the serum is taken, but only on those of other individuals; the stroma of the corpuscles cannot act as an antigen to the cells with which it comes normally into contact. (The way in which these auto-agglutinins are called into existence, and their meaning, if any, or use in the economy, are still unexplained.)

The existence and mode of formation of the secondary antibodies—*i.e.*, the antibodies to antibodies—point in the same direction. Thus, if we inject typhoid bacilli or their proteid constituents into rabbits or other animals, the specific antibody—the agglutinin—is produced, and accumulates in the blood in considerable amount, but it does not produce anti-agglutinin. If, however, we inject this serum into an animal of another species, and preferably one far removed from the first zoologically, the production of anti-agglutinin occurs. The agglutinin produced in any one species of animal must have, in addition to its peculiar clumping properties, the general constitution of a proteid characteristic of that animal, and hence be devoid of the power of forming antibodies whilst in the blood where it was produced. Similar phenomena occur in the production of other hæmolysins (amboceptor), which will be referred to subsequently.

With regard to the second criterion—the possession of haptophores which fit the receptors of the protoplasm of the animal into which it is injected—it is only necessary to say that, as far as can be traced, the substances which produce antibodies do, as a matter of fact, disappear from the blood of the animal in a short time—a few hours or days. As has been pointed out, the converse of this is not necessarily true; the toxin may disappear from the blood, and yet no antitoxin be formed, as in the case of tetanus toxin in scorpions. Here we have assumed that the scorpion contains naturally a proteid with a haptophore closely allied to that of tetanus toxin, that the toxophore of the latter is without action on the cell with which it is linked, and that the latter can use the former as pabulum in the same way as it uses the molecules of normal occurrence in its blood.

The third criterion is that the proteid molecule shall not be



capable of assimilation by the protoplasm to which it is anchored; it is not necessary that there should be a toxophore group which injures the cell. In this case the production of the antibody will not be accompanied by any symptoms of disease. The protoplasmic molecule will have some of its receptors occupied and rendered useless by the foreign proteid, and will have to regenerate others of the same nature, but it will not be injured in any way in the process. It is in this way that we explain the formation of antitoxins as a sequence to the injection of toxoids which have retained their haptophore groups, but lost their toxophores, as well as the formation of agglutinins (to non-pathogenic bacteria), precipitins, etc.

We pass on to another question—that of *specificity*. We have seen that the antitoxins are, in general, adapted only to neutralize the toxins which lead to their production. To this rule there are a few exceptions, real or apparent. Thus, anti-robin serum has also an action against ricin, tetanus antitoxin has apparently some action on snake-venom, anti-snake venom serum neutralizes scorpion venom, etc. The explanation is doubtless that the venoms in question have haptophore groups which closely resemble one another in their chemical affinities, whilst differing from those of other toxins. Thus, Ehrlich has suggested that robin is the toxon of ricin, in which case the combining portions of the molecules of the two would be identical.

In any case, the side-chain theory seems to fit quite well with ascertained facts. It explains the specificity; it is the receptors which unite with the injected toxin which are produced in excess, and which therefore form antitoxin. But in the complex changes in metabolism which must take place in the poisoned cell it is quite easy to imagine that, under certain circumstances, other receptors may either be *formed* in slight excess as a result of the general stimulation of the cell, or may be *cast off* in slight excess as a result of necrotic or autolytic processes taking place therein. This may be the explanation of some of the apparent exceptions, especially of those in which the serum has but slight antitoxic power on the toxin which was not injected, whereas it is much more potent on that which was.

The next question, and a much more difficult and important one, deals with the *site of production* of the antibodies. Ehrlich's original idea was that the antitoxins are produced from those cells on which the toxins act—*i.e.*, on the susceptible cells. For instance,

in the case of tetanus, he supposed that antitoxin is formed by the cells in the central nervous system, and explained the great difficulty of immunizing animals to this toxin by pointing out the enormous susceptibility of the cells to the action of this poison; it is only in the cells of the central nervous system that antitoxin can be formed, and these cells are extremely easily killed by the toxin, and are necessary for life. The chief evidence in favour of this theory is derived from the experiments of Wassermann and of Römer, which we shall now consider seriatim.

Wassermann's experiment may be regarded as a corollary to that of Ransom, who found that, after injection of tetanus toxin in pigeons (which are, in the ordinary sense of the word, insusceptible to that substance), he could extract it from all substances except the brain. Wassermann and Takaki found that the mixture of tetanus toxin with an emulsion of the central nervous system was no longer toxic when injected into susceptible animals; in other words, that the emulsion of central nervous system behaved like antitoxin. Emulsions of other organs are devoid of this power. They will combine with tetanus toxin, but will yield it again when injected into susceptible animals or macerated in salt solution. It appears, therefore, that the central nervous system is the seat, and the only seat, of an antitoxin-like substance, and it is thus rendered at least probable that when antitoxin appears in the blood it is due to its release from the cells which contain it normally—in other words, from the cells of the central nervous system, the cells which it attacks. The case of tetanus is the only one in which a toxin appears to have a definite selective influence on one group of cells, and is therefore unique in providing a means whereby Ehrlich's supposition as to the origin of antitoxin may be tested. It is no wonder that Wassermann's experiment has been submitted to an extraordinarily careful investigation, the results of which we must outline briefly. Some of these experiments seem to point to the truth of Ehrlich's assumption. Thus, Blumenthal showed that after injection of tetanus toxin into living animals the spinal cord lost its property of fixing the toxin *in vitro*, the receptors of the cells being already occupied with that substance. Further, boiled brain substance loses its power of neutralizing toxin, just as antitoxin does on being heated. He also showed that the central nervous system of fowls, which are but slightly susceptible to tetanus, has but little power of binding that toxin, so that emul-

sions of the brain must remain in contact with the toxin for some time before the latter is neutralized.

It was urged by many writers that the brain cannot be the site of production of antitoxin, since the injection of tetanus toxin direct into the brain of immunized animals causes the development of tetanic symptoms. This argument is obviously fallacious. A cell that is secreting antitoxin still possesses side-chains suitable for union with the toxin; it possesses them, indeed, in increased amount, and is therefore, if anything, more susceptible to the action of the toxin. The antitoxin which is circulating in the blood only prevents the toxin from reaching the cell, acting much in the same way as a lightning conductor.

Further research, however, seems to prove definitely that the neutralizing properties of brain substance are not due to the presence of a substance having any real likeness to antitoxin. Thus, Metchnikoff showed that when an emulsion of frog's brain is mixed with tetanus toxin no neutralization takes place, although the frog is, under certain circumstances, susceptible to the action of the toxin; these researches were corroborated by Courmont and Doyon. Arguing from this and other facts, Metchnikoff attributed the fixation to tetanus antitoxin by the central nervous system to the presence in the latter of fatty substances; these are absent from the brain of the frog. In support of this view, several observers found that substances such as lecithin, cholesterin, tyrosin, etc., have also the power of neutralizing various toxins.

It was objected to these researches of Metchnikoff and Courmont and Doyon that these experimenters did not leave the tetanus toxin in contact with the brain substance for a sufficiently long time. But this seems unnecessary, since the emulsion of the central nervous system of higher animals will neutralize tetanus toxin if the mixture be injected immediately, or even if the two substances are injected separately, though in this case the necessary amount of brain substance is larger. And Danysz showed that if a neutral mixture of brain and toxin be macerated for a *long* time in salt solution the toxin is again set free, in which again the phenomenon is unlike that presented by antitoxin in its action on toxin.

The researches of Morax and Marie also point in the same direction. They showed that the fixative power of the brain is almost entirely destroyed by drying, whereas, as we know, that process has practically no effect on antitoxin. The researches of

Dmitrevsky are also of great interest, and almost constitute a crucial experiment.

If Ehrlich's theory is true, and if antitoxin is produced by the budding-off of the receptors in great numbers, it ought to follow that the brain of an immunized animal, which contains these receptors in abnormally great amount, ought to have a greater power of neutralizing toxin than that of normal animals. But Dmitrevsky's experiments show that this is not the case, and constitute a strong proof against the theory in its original form: It seems fair to conclude that Wassermann's phenomenon is due to some accidental property of the central nervous system, or possibly to the presence of fats, and must not be quoted as evidence of the origin of antitoxin from the cells on which it chiefly acts.

Römer's experiment was made with abrin, a substance which has the power of causing violent conjunctivitis. He made use of rabbits, instilling small but increasing amounts into the right conjunctiva. After three weeks the animal was killed, and each conjunctiva dissected off, ground up with one lethal dose of abrin, and injected into animals. The right conjunctiva, that into which the abrin had been instilled, was found to act as an antitoxin and to neutralize the abrin, whereas the left was devoid of this power. Of course, if the process were carried on for a long time the abrin would be absorbed into the system, and there would be a *general* production of antitoxin, but at first the process appears local.

These researches are interesting, but do not seem to have any very direct bearing on the question at issue. They show—what does not require proof—that tissues which are not reached by the toxin do not produce antitoxin; but the question as to whether the latter substance is produced by the cells which are peculiarly susceptible to the toxin is not elucidated. There are in the conjunctiva numerous structures—connective-tissue cells, blood-vessels, endothelium, epithelium, leucocytes, etc. — and these experiments afford no means of gauging which of these are affected by the toxin or which produce antitoxin. The behaviour of the leucocytes is of especial interest; they are present in the inflamed conjunctiva in increased amounts, and the question may be asked whether the apparent antitoxic action of the right conjunctiva may not be due to these cells, which are present in but small numbers in the left. This view is rendered more probable from the further researches of Römer, who showed that in immunized animals antiabrin is present in greater amounts in the

organs rich in leucocytes, such as the spleen, lymph glands, and bone-marrow. This view harmonizes well with that of Metchnikoff, which we shall consider subsequently.

The chief arguments against the origin of antibodies from the cells which are especially acted on are those of Metchnikoff on the production of antispermotoxin, and from the study of other cytotoxins. The first series of experiments are of fundamental importance, and will be discussed here, although the substances in question are not simple bodies like toxins, but have a much more complex structure. The main facts are as follows: The injection of spermatozoa into living animals is followed by the production and appearance in the serum of certain substances which have the power of immobilizing and clumping the spermatozoa of the species from which the spermatozoa used for the injection was taken; we may call the substances spermotoxin. If the serum of animals which has been prepared in this way is now injected into animals of another species, the result will be the formation of an antispermotoxin analogous with antitoxin, which inhibits the action of the spermotoxic serum *in vitro* on the spermatozoa.

Now on Ehrlich's theory we may assume that this antispermotoxin is derived from the cells from which the spermotoxin is derived—*i.e.*, from the spermatozoa. But Metchnikoff proved that this is not the case, since the substance is developed as the result of the injection of spermotoxic serum into castrated males or into young animals of either sex. Antispermotoxins, therefore, are not necessarily derived from sperm cells. This is certainly a very striking result, and one that tells against the side-chain theory, but it is not conclusive for this reason—the cytotoxins are not usually sharply specific. Thus a cytotoxic serum made by the injection of kidney cells has a profound action on the kidney, but it usually has some hæmolytic action also, and may affect other cells as well. There do not seem to be any experiments bearing on the point, but it is at least probable that spermotoxins have some action on cells other than spermatozoa, and if this is the case Metchnikoff's experiments, considered as a proof against the side-chain theory, fall to the ground.

The mode of formation of the antihæmolysins also calls for short notice in this connection. The hæmolysins are, in some cases at least, more sharply specific than some of the cytotoxins, acting apparently almost exclusively on the red blood-corpuscles. It should follow on Ehrlich's theory that the antihæmolysins are

derived mainly from the red blood-corpuscles. This view, however, seems very difficult to believe when we consider that these substances are devoid of protoplasm and nuclei, and do not present any of those appearances indicative of active metabolism or secretion which we should expect in the case of a cell performing so profound a physiological function as the production of an antitoxin.

Nor is there any evidence, except in the one doubtful case of the action of brain substance on tetanus antitoxin, of the presence of substances like antibodies in the normal tissues and organs, in spite of the fact that they must contain receptors which would neutralize the toxins to which they are tested. Thus Calmette did not find that emulsions of brain possessed any neutralizing action on snake venom, although that substance certainly acts on the central nervous system, and there are many similar researches on the actions of other emulsions of organs on other toxins, all with negative results. We may quote, for example, those of Blum, who submitted various organs of the horse—liver, spleen, etc., all of which are certainly acted on by diphtheria toxin, since they show definite morphological lesions in acute cases of fatal intoxication—to prolonged autolysis, and found nothing in the nature of an antitoxin for diphtheria or snake venom in the resulting material. In one case—that of autolyzed lymphatic glands of the calf—he found a substance which reacted like tetanus antitoxin.

On the whole, therefore, it would seem that there is no good evidence for the theory that the cells which are acutely and powerfully poisoned by means of toxin are those from which the antitoxin is derived. It is certain, however, that this substance must be formed from cells which are reached and affected in some way by the toxin. The cells which especially require discussion are those of the connective tissues and the leucocytes.

Attention is drawn especially to the connective tissues from the fact that the most powerful production of antitoxin is usually elicited by a subcutaneous injection of toxin; intraperitoneal injection is in general less potent, and intravenous injection less still, and may not be followed by any production of antitoxin at all. The same law holds, and even more strongly, in the production of the other antibodies, although some exceptions occur.

Now in some cases it is true that these toxins have a local pathogenic action on the tissues into which they are injected, but

in others this is extremely slight, or not more than that due to the injection of any so-called "inert" fluid into that situation. Consider in this connection the reactions of certain animals to tetanus toxins. When this substance is injected into guinea-pigs, the effect is practically the same whether the injection is subcutaneous or intracerebral. In rabbits, on the other hand, a very much smaller dose suffices to kill if injected into the brain than is necessary if the injection is subcutaneous. Now rabbits are less susceptible to tetanus toxin than are guinea-pigs, requiring relatively larger doses to bring about a fatal issue. On the other hand, it is easier to prepare tetanus antitoxin from rabbits than from guinea-pigs by the injection of tetanus toxin, though difficult in both cases. Compare these results with those obtained from fowls. These animals are resistant against tetanus toxin (except in absolutely enormous doses) when injected subcutaneously, but are easily affected when the injection is made into the brain or subarachnoid space. The probable explanation turns on the varying affinity of the toxin for different parts of the body. We may assume in all cases that the tetanus toxin acts as a poison only on the brain and spinal cord, and that in the guinea-pigs it has practically no affinity for the subcutaneous tissues, and that when injected into this situation it passes ultimately to the brain almost without loss. In rabbits some of the toxin is united to the tissues, and only a part reaches the central nervous system, whilst in fowls the affinity of the tissues for the toxin is so great that the whole of the latter is absorbed and the brain escapes injury. Now it is practically impossible to obtain antitoxin by injecting toxin into the guinea-pig, difficult to obtain it from the rabbit, but easy to obtain it from the fowl, so that it would appear that it is the tissues which are not especially vulnerable to the toxin which yield it. If this is true, it is no argument against the validity of the side-chain theory. It simply indicates that the cells which are profoundly affected with toxin are thereby rendered unable to produce antitoxin in virtue of the toxic action of the poison, the other conditions being suitable. We know this to be the case from the fact that we can produce tetanus antitoxin from guinea-pigs as a result of the injection of toxoid, which we must assume to unite entirely with the cells of the central nervous system, just as the toxin does, though without injuring them. We may assume, therefore, that antitoxin is produced from cells which unite with toxin or toxoid, but which

are not too profoundly injured thereby to perform their functions of self-nutrition in a normal manner.

The question whether we can narrow down the issue to one particular set of cells which always occur in the connective and other tissues—to wit, the leucocytes—is a more difficult one. We have already adverted to the researches of Römer, who found that antiabrin was present in immunized animals in greatest amount in the organs that are rich in leucocytes, and Metchnikoff has brought forward a whole series of researches which point strongly in the same direction. The discussion of some of these will be deferred to a subsequent chapter, since they hardly seem to indicate that the leucocytes form antitoxin, though they do go to prove that they discharge another and equally important function in the production of immunity to toxins. Here it will only be necessary to point out some well-known and important facts which appear to have a bearing on the question of the origin of antitoxin. We allude especially to the chemotactic attraction for leucocytes which is shown by almost all toxins when not present in too great an amount. In virtue of this attraction the most marked and constant feature of intoxication is the presence of an excess of leucocytes, and this is, in general, most marked when there is a balanced contest between the toxin and the host—*i.e.*, when the latter is neither destroyed at once nor shows but little effect of the injection. It is practically a general rule that wherever the host is victorious in the struggle with the toxin there is an excess of leucocytes; and even where the fatal issue is not averted there is some leucocytosis, unless the intoxication is a very grave one and very rapidly fatal. This is well known in human pathology in the infective processes, where a diminution of the number of leucocytes is a bad sign, whereas hyperleucocytosis is a good sign in so far as it indicates that the infection, though intense, is being combated by the patient by the best means at his disposal. Many researches have indicated that the same law holds in these intoxications. Thus Chatenay, in an exhaustive study on the reactions of the more important toxins, finds that a dose that is rapidly fatal brings about a fall in the leucocytes, whereas smaller doses cause hyperleucocytosis; thus, in the guinea-pig about 100 lethal doses are required to prevent the occurrence of the latter. Now we can scarcely imagine that so widespread and constant a phenomenon is without some meaning or without some use to the



organism, and since it always occurs and is most marked under conditions in which antitoxin is formed, it is highly probable that the leucocytes may be, entirely or in part, the origin of the antitoxin. But we must not forget that in many cases the leucocytes play other parts in the struggle against the infections; for example, it is plausible to argue that their presence in excess in infected areas is an attempt to combat the bacteria themselves, and not the toxins which they produce. In this case their access to areas into which a toxin had been injected might be useless, and they might simply be attracted there under the mistaken supposition that there would be bacteria to combat as well as toxins. But this view is rendered improbable in face of the numerous facts that have been brought out by the French school with regard to the absorption of toxins and other poisons by the leucocytes. Thus Metchnikoff showed (and it is a fact of the utmost importance) that if an aseptic exudate be produced in a fowl that has been injected with tetanus toxin, that substance can be demonstrated in large amount in the leucocytes of the exudate. Again, Vaillard and Vincent have shown that if tetanus bacilli or spores washed free from toxin are injected into a guinea-pig, they become surrounded by leucocytes, and that under such circumstances no tetanic symptoms occur.

With regard to the action of other poisons the facts are still more striking. Thus rabbits are far more susceptible to the intracerebral injection of atropin than to the same substance injected into the circulation; and when the injection has been made in the second manner, atropin can be demonstrated from the leucocytes, whereas it is not present, or only in very small amounts, in the plasma and red corpuscles. Similar facts have been found for arsenic and other poisons. Still more striking are the researches of Besredka with regard to arsenic. He studied first the action of the trisulphide of arsenic, an almost insoluble substance, yet very poisonous. When this was injected in small amounts into the peritoneal cavity of a guinea-pig, there was a marked increase in the mononuclear leucocytes in that situation, and in a short time these cells had ingested the whole of the arsenic, which could be recognized as fine granules in their protoplasm. These gradually became smaller and ultimately disappeared, being probably converted into a non-toxic compound—perhaps by some process analogous with antitoxin formation. In a second series of experiments he tested the action of the

same substance when shielded from the leucocytes by being enclosed in permeable bags before being placed in the peritoneum; in this case the animal died, the arsenic doubtless becoming dissolved, and thus escaping the action of the leucocytes. He found also that substances which diminished the number of the leucocytes in the peritoneal fluid aided the action of the arsenic, whilst those which caused a leucocytosis diminished it. He found similar facts with relation to the action of a soluble salt of arsenic, and showed that in animals which had been immunized to that substance the arsenic is especially taken up by the leucocytes. There can be no doubt, therefore, that the leucocytes play a rôle of the utmost importance in the defence of the body against the ordinary poisons.

All things being considered, we may deduce that antitoxin is formed from any cell with which the toxin can combine, provided that that cell is not too profoundly injured in the process, and may attribute to the leucocytes an important, though not an exclusive, rôle in this process. It is hardly necessary to say that this does not disprove the side-chain theory; we must regard the leucocytes as being cells which are specially told off for the defence of the organism against infections and intoxications. They are, in consequence, specially immune to the action of toxins, being able to resist amounts which are injurious to the more highly organized tissues; that this is the case is proved by their invasion and persistence in a living state in areas in which the tissues are profoundly injured by a toxin. It is not difficult to believe that these resistant cells, which have a special predilection for intoxicated areas in which antitoxin is especially required, should be the source of that substance. We have, then, only to regard them as possessing suitable haptophore groups and as being relatively insusceptible to the toxophore groups—both of which are inherently probable—to see that they fulfil in a special way the criteria which the side-chain theory demands for cells which are to produce antitoxin when exposed to the action of toxins.

## CHAPTER VI

### IMMUNITY TO TOXINS

THE mechanisms by which the injurious effects of toxin in the blood and tissues are combated are exceedingly complex, and may be divided roughly into two series. In the first and less interesting, though perhaps not less important, the mechanism may be called (for want of a better term) non-specific. It acts equally on all or many toxins, and recovery from the effects of the poison is not necessarily or usually followed by any immunity thereto. For example, the dilatation of the vessels and acceleration of the blood-stream which takes place in an inflamed focus may be regarded as one of the natural provisions for avoiding the too intense action of a toxin in one small area. The poison produced is rapidly swept into, and diluted by, the whole blood-stream, and if not produced in large amounts may not reach any tissue in amount sufficient to cause much damage. Perhaps, also, the proteolytic action of the enzymes frequently produced (mainly from the polynuclear leucocytes) in the inflammatory focus are of some importance. Toxins are very fragile bodies, and it is extremely probable that the potent enzymes which occur in all fluids rich in polynuclear leucocytes may bring about their destruction in considerable amounts.

The more purely chemical processes are hardly known in their application to true toxins, but they have been largely studied in regard to the methods by which the organism combats deleterious substances produced in the course of metabolism, or absorbed from the alimentary canal. The latter are for the most part bacterial products, though not true toxins. It is, however, quite possible that these latter substances are destroyed by the same processes, which Herter classifies as (1) oxidation, (2) hydration and dehydration, and (3) various syntheses.

As an example of the process of oxidation, we may consider the formation of indol, a toxic substance, which is formed by *B. coli*

and other organisms in the alimentary canal, and which probably plays some part in the production of the symptoms of auto-intoxication due to abnormal digestive processes occurring in the small intestine. It undergoes oxidation to indoxyl, a much less toxic substance, which then combines with sulphate of potash to form indoxyl-potassium-sulphate, or indican. There is reason to believe that this process takes place to a very large extent in the liver, one of the chief functions of which is the destruction or neutralization of poisonous substances conveyed to it by the portal circulation. Thus the toxic dose of nicotin and of some other alkaloids is much less when the injection is made into the general circulation than if the substance be injected into the portal vein. This, it must be noticed, is not due merely to the elimination of the poison in the bile, though that may happen. The liver cells have the power of actually destroying certain of the alkaloids. Thus hyoscyamine mixed with surviving liver pulp is rapidly destroyed. In the same way, indol, phenol, and skatol, all toxic substances of bacterial origin, cannot be recovered by distillation after contact with liver cells. It appears, therefore, that much of the immunity to non-specific poisons is dependent on the functional activity of this organ, which is, so to speak, interpolated in the blood-current in order that it may rid the circulation of certain poisons, and that these are eliminated in the bile or submitted to various chemical modifications, in the course of which they become inert, or at least less toxic.

Examples of the neutralization of poisons in the body are numerous, the most important being, perhaps, the formation of urea, a relatively non-toxic substance, by the synthesis of ammonia and carbonic acid, followed by dehydration, this process taking place mainly or entirely in the liver. Other syntheses, however, take place in other organs—for example, the union of glycocholic and benzoic acid is performed in the kidney. And we shall see subsequently that the leucocytes and leucocytic organs have a very special and important duty to perform in dealing with poisons of all sorts, both organic and inorganic, though the exact chemical processes that they bring about are unknown. It will be more convenient to defer the consideration of these cells for the present.

The exact application of these “non-specific” processes of detoxication to the bacterial toxins has not yet been worked out, but there can be little doubt of its great importance. Compare,

for example, the severity of the symptoms due to an abscess due to *B. coli* and the lack of symptoms due to the absorption of the toxins of this organism which occurs from the alimentary canal, and very probably from bacilli which actually make their way through its walls into the lacteals. There can be but little doubt that the symptoms of intoxication in the former case are due to the direct access of the bacterial poisons into the blood-stream without having first to traverse the liver. Again, in a case of diphtheria of moderate severity virulent bacilli are often present in abundance so great, that by comparison with the toxin-forming powers of the organisms *in vitro* we might expect a rapidly fatal issue from the toxin absorbed, and yet the symptoms of general intoxication may not be severe. In all probability only a small fraction of the toxin formed actually reaches the distant tissues. Some may be dealt with by the zone of leucocytes which underlies the layer of diphtheria bacilli, but it is also possible that some of the toxin is destroyed in the blood-stream, perhaps by a process of oxidation. These non-specific methods of dealing with toxins are matters more for the physiologist than for the pathologist, depending as they do simply on the perfect discharge of the normal functions of the body. They are of great importance—greater, perhaps, than pathologists usually realize, the interest attaching to them being so much less than that which is connected with the study of the antitoxins and similar bodies. They are to be regarded as the first line of defence against poisons of all sorts. When a *small* dose of a specific bacterial toxin gains access it is probable that the natural physiological methods in daily use for dealing with the natural poisons (formed in metabolism or absorbed from the alimentary canal) are in most cases applicable to it also, and no specific process has to be brought into action. In confirmation of this view is the fact that diphtheria antitoxin is not necessarily found in the blood after natural recovery from diphtheria, suggesting that in these cases the processes at work are non-specific.

But when the toxin is present in greater amount this process may fail, and it may do so in consequence of the action of the poison on the tissues and organs normally concerned in the defence of the body. The neutralization of toxins, etc., in the liver and other regions can be carried out best when these organs are in a state of high functional activity, and when this is impaired some of the deleterious substances will escape their action. Thus we

get a vicious circle. The poison injures the liver or other organ and impairs its defensive powers. More poison is allowed to circulate in the blood, and the liver is injured still more. When this occurs we are thrown back on the specific methods of dealing with the toxins, which take longer to come into action, and which may be regarded as the second line of defence. In one case, however, we must regard them as of chief importance. Thus in tetanus the toxin is produced locally, and ascends the nerve-trunks without entering the blood-stream or being carried to the liver or leucocytic organs. Here, then, in all probability, specific methods have to be resorted to, and the chance of recovery depends on their early development.

The question of specific immunity to toxins, and of recovery from intoxication with true toxins, may be considered under three heads:

1. Acquired immunity, due to disease or to vaccination with toxins or toxoids (chemical vaccination).
2. Antitoxic or passive immunity, due to the injection of antitoxic serum, or possibly to its natural occurrence in the blood.
3. Natural immunity, not due to vaccination, and not accompanied by antitoxin in the blood.

It will be convenient to consider them in this order.

Natural recovery from toxic diseases such as diphtheria and tetanus is not necessarily accompanied by the appearance of antitoxin in the blood—at least, not in demonstrable amounts. Further, it appears from the researches of Abel that antitoxin, when it is formed at all, does not make its appearance until about the eighth day of the disease, at which period the severity of the toxic symptoms may have already begun to decline. What may be called the simple antitoxic theory cannot be maintained. Under natural conditions the process of recovery is not due simply to the production of antitoxin, though it is possible that this comes into action in prolonged cases.

Further, acquired immunity to toxins is not due solely to the presence of antitoxin in the blood. If it were so it should develop proportionately to the development of antitoxin, and the two should persist for the same length of time, and disappear together. This, however, is not the case. We have already seen that animals in the early stage of immunization to diphtheria and tetanus frequently present a decreased amount of resistance, or hypersensitiveness, to these substances, and may die with symptoms of acute intoxica-

tion, in spite of the presence in the blood of amounts of antitoxin sufficient to neutralize the toxin many times over. Similar appearances may be seen in animals in which death from acute intoxication does not occur. Thus, in two horses which were treated at the same time for the production of diphtheria antitoxin, one showed but little sign of the action of the toxin, and improved in general health during the treatment—it developed only 5 units of antitoxin per cubic centimetre of serum; the other suffered severely in general health, and had ultimately to be killed—it developed 70 units per cubic centimetre. Similar phenomena are often met with, and, as a general rule (to which there are numerous exceptions), the presence of a large amount of antitoxin in the blood indicates susceptibility rather than immunity. The facts of the later stages of antitoxin-formation may also be borne in mind. Sooner or later in the history of any antitoxin horse there comes a time when the amount of antitoxin begins to diminish, and would, in all probability, disappear entirely if the injections were continued; yet these horses are extremely resistant to the action of toxin—more so, in fact, than animals with much antitoxin in the blood. The degree of immunity, therefore, is not measured by the amount of antitoxin in the blood, and we might argue that the two are not related in any way. This, however, is absurd, in view of the ascertained action of antitoxin in neutralizing the effects of toxin *in vitro*, or of protecting against a dose surely fatal. We may consider the rôle of antitoxin under two heads: (1) in immunity, and (2) in recovery from disease.

Considering first the question why an animal dies in spite of the presence of an excess of antitoxin in the blood, we must regard the simplest and most probable explanation as one on which the toxin-antitoxin molecule is looked upon as dissociable. This is always the case on Arrhenius and Madsen's theory of the interaction of the two substances, whilst on the colloid theory the dissociation only takes place for a short time after the compound has formed, the union between the two substances gradually becoming firmer and firmer. On this supposition the failure of antitoxin is readily explicable: the two substances unite, and the inert molecule is formed; this dissociates, and the toxin-molecules, which happen to be set free in the neighbourhood of susceptible cells, unites with them. The removal of some of the molecules of toxin allows more dissociation to take place, and ultimately the whole of this substance is passed on to the tissues. We must assume that

the compound formed between the toxin and the tissues does not dissociate, as, indeed, appears probable.

But if this is the case, the effect of antitoxin in the blood would be merely to delay the action of the toxin; assuming this, how are we to explain the preventive effect of this substance in passive immunity and its curative effect in disease? For it is only in comparatively rare cases in the early stages of antitoxin formation that the phenomena under discussion occur, and in all other conditions the presence of a sufficient amount of antitoxin in the blood constitutes a perfect safeguard against the action of its corresponding toxin. It is probable that *the leucocyte* is the all-important factor necessary for the destruction of these specific toxins, whether previously neutralized by antitoxin or not. We must look upon the toxin-antitoxin molecule as one which can be easily ingested and destroyed by the leucocytes, and the neutralization of toxin by antitoxin as the first step in a double process, the second being the destruction of the compound by the leucocytes. If antitoxin is absent, the leucocytes may still deal successfully with the toxin, if the latter be not too virulent, nor present in too large an amount; but if the leucocytes make default, the presence of antitoxin may delay the lethal issue, though it is powerless to avert it. We may, perhaps, compare antitoxin with opsonin, which unites with bacteria and renders them suitable for ingestion by the white corpuscles. Metchnikoff and his school have paid great attention to the rôle of the leucocytes in intoxication, and have brought forward very important and suggestive evidence, pointing to the white corpuscles, and especially the large lymphocytes (macrophages) as the main source of antitoxin. This question is dealt with elsewhere, and at present we have to discuss only the rôle of the leucocytes in dealing with the toxins, either alone or when neutralized by antitoxin.

The evidence proving the importance of the leucocytes in dealing with unaltered toxins is abundant, and some of the more striking facts brought forward by the French school have been briefly summarized in the last chapter. The evidence for the removal of toxin in combination with antitoxin by leucocytic action is less direct, since it is obviously impossible to recognize this compound by chemical or microscopical processes whilst in the leucocyte. But it has been shown that where toxins are introduced in combination with solid substances, such as carmine or brain tissue, they become surrounded by large numbers of leucocytes, and the



particles are taken up by these cells. Further, the injection of toxin into an animal which already contains antitoxin in the blood brings about a more or less marked leucocytosis. It is true that the local leucocytosis brought about by the injection of neutral mixtures of the two may be but slight, but we must remember that substances in a state of solution are quickly absorbed and carried to the lymph glands or bloodvessels, in either of which situations leucocytes occur in plenty. Further, there are always some leucocytes in the tissues, and the number may be sufficient to deal with the amount of toxin-antitoxin injected, which, considered as mere weight, is always very small.

The effect of antitoxin on the toxin of *B. pyocyaneus* may also be cited as a phenomenon capable of explanation on this supposition. A few lethal doses of toxin are fully neutralized by antitoxin, the law of multiple proportions holding up to a certain point. When, however, more than ten lethal doses are injected the law does not hold, and no amount of antitoxin will avert the fatal issue. Here we must assume that the antitoxin has but a slight affinity for the toxin, so that dissociation takes place rapidly, and the ultimate cause of the destruction of the toxin to be leucocytic activity. The single lethal dose is just more than the leucocytes can deal with; but when antitoxin is injected simultaneously the leucocytes have time to come into action, and the toxin, gradually set free by dissociation, is dealt with in detail instead of in a single dose. There are, however, limits to this process, and when more than ten lethal doses are present fully combined with toxin we must imagine that dissociation goes on so rapidly that more toxin than one lethal dose is set free before the toxin-antitoxin molecules can be taken up. If this explanation is highly theoretical, it seems the best that is available at present.

The difference between the effect of toxin in animals with antitoxin in the blood, but otherwise normal, and its effect in animals in the early stage of antitoxin formation, is explicable if we regard the hypersensitiveness as occurring in the leucocytes themselves as well as in the tissues. When antitoxin is injected into a normal animal, the leucocytes of which are functionally active, the conditions for the immediate destruction of the inert molecule are present, and no dissociated toxin gains access to the susceptible tissues. But in hypersensitive animals the leucocytes will be unable to deal with the molecule, or will perhaps be killed by toxin liberated by dissociation taking place within their protoplasm, and

the toxin will ultimately reach the tissues. And it is probable that the beneficial effect of early as compared with late injections of diphtheria toxin may be dependent in part on the fact that in this disease the leucocytes are very apt to suffer degenerative changes which doubtless impair their defensive powers. Ewing believes that the variations in the staining capacity of these cells (loss of chromatin being an early and easily recognized sign of their degeneration) might be utilized as a means of prognosis, and points out that in cases which recover the staining quality of many of the leucocytes undergoes a rapid improvement after the injection of antitoxin, whereas in fatal cases this change could not be detected. As a rule the total number of leucocytes is diminished immediately after the injection of antitoxin, but in some fatal cases the previously existing leucocytosis may remain or even increase. This fall in the total number of leucocytes is probably to be accounted for by the neutralization of the toxin, so that fresh white corpuscles are no longer attracted from the bone-marrow by positive chemotaxis, whilst the old and injured cells remain in the spleen or other internal organs, no longer circulating in the blood. In cases which die the reduction in the number of leucocytes may be followed by a subsequent rise.

Here also we must refer to numerous researches, emanating more especially from the French school, which go to show that substances which are certainly devoid of true specific antagonism have nevertheless the power of preventing or diminishing the action of toxins in virtue of causing an increased inflow of leucocytes to the region into which the injections are made. This question has been more especially investigated in regard to the effect of this artificially produced local leucocytosis in causing local bacterial immunity, but Calmette and Delecarde have shown that the peritoneal injections of ordinary broth cause a certain degree of immunity to abrin, whereas normal saline solution has no such power. The former substance is a powerful agent in attracting leucocytes, whilst the latter is much less potent in this respect, though by no means devoid of activity. Substances which have the power of increasing the action of the leucocytes in this way are sometimes termed "stimulins," but the word should be avoided. The action of leucocytes in any region may be increased by many methods, the chief of which are: (1) attracting larger numbers by means of substances having a positive chemotactic action; (2) stimulating the activity of the leucocytes; and

(3) altering the nature of the substances on which they are to act. If the term "stimulin" is to be used at all, it should be rigidly restricted to one that acts in the second way, and there is as yet no strict proof that such a body occurs. Substances acting in the third way are called opsonins, and, if the theories of antitoxic action here outlined are correct, antitoxins have this action also.

Turning now to the process of recovery from bacterial intoxications in natural disease, we find but little evidence that the formation of antitoxin plays any active part in the process. The conditions are not as a rule suitable for the production of this substance, since the constant flow of the toxin into the circulation would lead rather to a summation of negative phases and the production of hypersensitiveness. Further, the process of recovery may have commenced before antitoxin appears in the blood—a phenomenon which, when it occurs, we may regard rather as a means of preventing reinfection than as a mechanism for the cure of the original attack of the disease. (It may be pointed out that in the case of diphtheria the studies of Ruth Tunnicliffe lead us to believe that the process of recovery runs parallel to, and is due to a rise in, the opsonic index, and that the main factor in the cure of the disease is the removal of the bacilli by a process of phagocytosis, and consequent cessation of the absorption of toxin.) In prolonged diseases it is possible that the production, and especially, perhaps, the *local* production of antitoxin, may play some part in the process. In other cases, perhaps, the toxin is dealt with by some of the simpler chemical processes considered already and submitted to various changes of oxidation, etc., and thus rendered inert; but of this we have but little knowledge, the main studies of the methods in which the body deals with toxins having been devoted to the phenomena occurring in highly immunized animals and brought about by their sera.

Some experiments designed by Wassermann as evidence in support of the side-chain theory may be alluded to in this connection. He found that the injection of tetanus toxoids into highly susceptible animals (guinea-pigs) has the power of rendering them partially immune to tetanus toxin for a short period. Thus, when the toxoid was injected, followed in an hour's time by unaltered toxin, the lethal dose of the latter was greater than in a normal animal. On the other hand, after a day or two the animal became more susceptible, being killed by less than the minimal lethal dose for normal guinea-pigs. This he explained

by supposing that the receptors of the sensitive cells were occupied by the inert toxoid, so that part of the cells would be unable to combine with the true toxin. The subsequent susceptibility he explains by the over-production of new receptors. Now when we consider that in acute disease of natural occurrence the production of toxin (in cases that recover) is only temporary, and is stopped sooner or later by the destruction of the bacteria, it seems probable that some such process may occur and serve to shorten the period during which the cells are exposed to the action of the toxin. The true toxins are so fragile that it is highly probable that a certain proportion of them are converted into toxoids during or after the process of absorption into the blood-stream, and by occupying their receptors diminish the susceptibility of these cells to the true toxin, which, circulating in the blood, may be dealt with by the leucocytes, liver, or other organs. Afterwards anti-toxin would appear in the blood, but it would have no relation to the early slight immunity of the tissues.

We may therefore recognize the following stages in the process of recovery from a bacterial intoxication :

1. In the first the processes are mainly non-specific, the chief being probably leucocytic action ; the dilution of the toxin in the general blood-stream, and its elimination, either in a natural condition or after it has undergone some chemical alteration ; the partial destruction and conversion of toxin into toxoids, and subsequent " blocking " of side-chains in highly important tissues. In mild cases these may be sufficient for the restoration of health, and no immunity, or the merest trace, may follow.

2. Where the process lasts longer antitoxin begins to be produced, and in all probability the time which must elapse before this occurs varies greatly in different conditions, depending on : (a) the constitution of the patient, (b) the dose of the toxin, and (c) on the nature of the toxin. Thus, with regard to the last point, it may be remarked that it appears to be extremely difficult to prepare antitoxins to the endotoxins, and it is highly probable that in recovery from such diseases as cholera and typhoid fever the production of antitoxin is slight or absent. Such diseases owe their symptoms mainly to the production of endotoxin, and are combated mainly by the removal of the bacteria which produce them. In the cases in which antitoxin is produced, recovery at this stage depends on its presence, together with the functional efficiency and sufficient numbers of the leuco-

cytes, and the continuance of the factors by which the toxin is combated in the first stage of the infection.

Passive antitoxic immunity consists in the artificial production of this stage.

3. In the third stage, which is only reached in hyperimmunized animals submitted to treatment for long periods, the conditions are quite different, and do not appear to depend in any way on the action of antitoxin, but approach more nearly to the state of natural immunity to be treated subsequently. In this condition the antitoxin in the blood falls greatly, and would probably disappear entirely if the treatment were carried further, yet the degree of immunity is very great. There are two or three theories which have been invoked to explain this form.

In the first place, if we accept the side-chain theory, we may ascribe it to the absence of receptors suitable for the toxin which is being injected, and may suppose that the repeated and prolonged stimulation of the production of this particular receptor has led firstly to a hypertrophy, and secondly to an atrophy of these organs. This is readily conceivable, and might be compared with the sequence of hypertrophy and failure of the heart so frequently met with in Bright's disease, etc. In this case the toxin would be unable to "anchor" itself to the cells, which would thus escape its action, and the result would be one form of *tissue immunity*. There is but little experimental evidence bearing directly on this theory either for or against. The state is one rarely seen even under experimental conditions, though of great theoretical interest. It is found, however, that during the immunization of the rabbit with eel serum (a potent hæmolytic agent) an antitoxin or anti-hæmolysin is produced, whereas the corpuscles themselves, if carefully washed from all traces of serum, are as susceptible as before. If, however, the injections are repeated, the antitoxin disappears, and, as Tschistovitch showed, about this time the red corpuscles themselves become immune, not being hæmolyzed by eel serum, even although all traces of their own serum is washed out. This we may fairly suppose to be due to a loss of the receptors with which the molecules of eel serum combine, and this may be taken as an experimental demonstration of the occurrence of this form of immunity, which was predicted by Ehrlich on theoretical grounds. Metchnikoff, it is true, objects to this hypothesis, pointing out that if the receptors are so necessary for the nutrition of the molecule it is impossible that

these latter should continue to live after the receptors had been lost. But this seems based on a misconception of Ehrlich's theories of cell-nutrition. We must imagine each molecule to be provided with a very large number of varieties of side-chain, each adapted to the seizure of a different form of food-molecule, so that the complete loss of one variety does not imply that the cell will suffer in nutrition. The only result is that more work will be thrown on the receptors that remain. There is nothing improbable in this. It is in the highest degree likely that so important a function as nutrition should not be dependent on the functional integrity of every part of the molecule. The occurrence of tissue immunity from loss of receptors is at least possible.

An alternative theory asserts that the cells of the body retain their power of combining with toxin, but lose their susceptibility to the action of the toxophore group of the latter, at the same time losing their power of producing antitoxin. We may explain the latter phenomenon by saying that the receptors in question become sessile and incapable of being shed. There is experimental evidence that some receptors are naturally of this nature, and it is quite possible that ordinary deciduous receptors might change in this way. As we shall see, tissue immunity in which the toxin becomes anchored to the cell, but without injuring it or stimulating it to produce antitoxin, is met with in some forms of natural immunity, and it is quite possible that it may occur in the process of hyperimmunization by vaccines, though direct proof is lacking. Another process of somewhat similar nature may occur, which has also its analogue in natural immunity. Thus when animals are immunized to tetanus they still retain their susceptibility to that toxin if the injection be made directly into the brain. It is obvious, therefore, that the cells of the central nervous system are still susceptible. Why, then, do the animals not develop tetanus if the injections be made subcutaneously? We exclude, of course, the case in which antitoxin is present in the blood and the leucocytes are functionally active. It will be shown subsequently that some forms of natural immunity depend upon the fact that some cells in the body unite with the toxin, and have, indeed, a great affinity for it, but are not injured thereby, whereas others have an affinity for it, but are killed by its action. Obviously, if the toxin be injected into tissues of the former class it will be absorbed, none will reach the susceptible second class of cells, and no toxic symptoms will result. Now let us suppose

that in immunizing a susceptible animal (rabbit or guinea-pig) to tetanus the connective tissues acquire receptors suitable for the toxin in question. They will absorb this toxin, be shed, and the animal will develop antitoxin, whilst the tissues previously susceptible—the brain and cord—will remain as susceptible as before. This is probably what occurs, though the critical proof—the demonstration of an increase in the toxin-absorbing power of the tissues in acquired immunity, and thus of an increased number of receptors—does not appear to be forthcoming. Dmitrevsky investigated the amount of tetanus toxin neutralized by brain tissue before and after immunization; but since, as we have seen, this process is probably different in nature from the neutralization of toxin by antitoxin, his researches have not much weight in this connection.

The third explanation is that of Metchnikoff, that the leucocytes themselves have become immune to the toxin, and have thus acquired the power of dealing with that substance in large quantities, so that the tissues are sheltered from its action. This theory simply shifts the immunity back to the leucocytes, and thus does not really solve the problem, which, it must be admitted, is unsolved whatever theory we may adopt, and perhaps insoluble in the present state of our knowledge of the physical chemistry of living matter. We may, however, combine it with perfect propriety with the side-chain theory, and argue that the leucocytes may have behaved in the same way as we have supposed the tissue cells to have done in the last paragraph—that is, to have retained, and perhaps increased, their receptors, whilst losing their susceptibility to the toxophore group. Here, again, we are confronted with the absence of any definite evidence either way. There seems to be no experimental proof to show whether leucocytes from a hypervaccinated animal have any increased resistance to the poisonous action of toxins or any increased power of combining with them. This is greatly to be deplored, and might easily be remedied now that Sir Almroth Wright has taught us a simple and convenient method of working with living leucocytes. As far as our experimental knowledge goes, there is no great difference in leucocytes from immunized and normal animals, and the researches on opsonins have led to a tendency to disregard the possible variations in the immunity of the leucocytes, since the researches of Bulloch and others showed that leucocytes from very varied sources would take up the same number of bacteria

under identical conditions. Subsequently, as we have seen above, some slight indications of a difference have been found. But on Metchnikoff's theories there ought to be a very marked difference, and we should expect that, the leucocyte being *par excellence* the cell devoted to the protection of the animal body against infections, it would be the first to acquire increased resistance in acquired immunity, and we should expect, *e.g.*, leucocytes from a convalescent case of pneumonia or from an animal vaccinated against the pneumococcus to take up far more pneumococci than leucocytes from a normal person under similar conditions, whereas the difference, if one exists, is extremely slight. A careful consideration of the conditions of opsonic experiments leads to the conclusion that the results obtained are not to be regarded in any sense as an index of the immunity of the leucocytes. Ledingham has shown that the number of bacteria taken up by the leucocytes depends on the extent to which the former have been altered by the action of serum, and not on the temperature at which the phagocytosis takes place. Thus, if the bacteria are sensitized by serum, mixed with leucocytes, and part kept at 18° C. and part at 37° C., the number of leucocytes is the same in the two cases. Now at the lower temperature no active movements of the leucocytes take place, so that we cannot regard the ingestion of the bacteria as being entirely, as was formerly assumed, an active process brought about by a seizure or surrounding of the organism by pseudopodia, as can be seen so readily in the amœba. Such a process does occur to some extent, and can be seen to occur under suitable conditions; but it is also true that bacteria can be seen to make their way into a cell which has been watched continuously under a high power of the microscope, and in which no movement of any sort has been witnessed. We are led, therefore, to believe that the phagocytosis which occurs in opsonin experiments *in vitro* is a process allied to agglutination rather than to an actual physical seizing of an organism. It is one which can take place without an actual conscious—if we may use the term—movement of the leucocyte towards its prey. An immunized leucocyte would be immune to bacteria which it had ingested, to the endotoxins liberated in the process of bacteriolysis, whether taking place within the leucocyte or outside it, and to endotoxins; and it would exert its physiological functions of movement (pseudopodia-production and chemotaxis) equally well whether these toxins were present or absent. Or the effect



of a given toxin might be reversed in the immunized leucocytes, so that in place of being repelled, as under normal conditions, it might be attracted. As far as phagocytosis depends on the chemotactic attraction of leucocytes and the active suggestion of organisms, we might expect it to be greatly increased if these cells became immune. But there is no reason to see how a similar increase need occur if it depends on a process akin to agglutination, and resulting, perhaps, from a change in the surface tension between the leucocyte and the organism. And that this is the way in which the vast majority of the bacteria are taken up in ordinary opsonin preparations appears probable. Active movements of leucocytes are rarely seen in emulsions prepared for the opsonic technique and examined in normal saline solution on the warm stage. In other words, the opsonic index, though useful as an index of certain activities of the serum, would appear not to throw much, if any, light on the immunity of the leucocytes. That must be sought by other means, notably by experiments *in vivo*, and these lead us to the belief that the leucocytes may become immune and may play a part of importance in acquired immunity.

But the facts which have been previously recorded concerning the local reaction in animals which are being immunized to tetanus toxin tend to support Metchnikoff's hypothesis, though the evidence is somewhat indirect. We have already pointed out that when normal horses are injected with small doses of this toxin there is at first no local reaction, or but slight, and that as the animal becomes more immune this local reaction becomes more manifest—a phenomenon which we have attributed to the hypersensitiveness of the tissues. Now the local reaction is inflammatory in nature, and the tumefaction which occurs is due partly to oedema and partly to an access of leucocytes, so that it would appear that in the production of immunity these cells acquired the power of invading a tissue which is permeated with toxin. If this is the case, we may assume that they have become immunized to this toxin, and that they have changed in regard to their chemotactic properties. This collection of leucocytes in large numbers at the region of inoculation of toxins, where they may form a *sterile* abscess of considerable extent, is a frequent phenomenon in the production of diphtheria toxin; and here, again, it seems not improbable that the leucocytes have become, like the tissue cells, more immune to the toxin. There are no inherent

difficulties in the supposition, and it certainly favours the explanation of the phenomenon.

It will be remarked that the tendency of modern thought has been rather to minimize the importance of antitoxin in the process of natural recovery and in the subsequent immunity, and to regard its appearance rather as an epiphenomenon, though doubtless one that may under certain circumstances be of advantage to the patient. Let us consider briefly the probable significance of antitoxin-formation in the animal economy. Is it a purposive phenomenon, developed and selected by natural selection with a view to defend the species against natural dangers? This was the natural assumption when antitoxin was thought to play a rôle of the utmost importance in natural recovery and acquired immunity. There are great difficulties in the way of accepting such a hypothesis. In the first place, immunity to bacterial infections is more prevalent in the lower rather than in the higher animal types. Doubtless very great susceptibility to a widespread infective agent would operate unfavourably to the prospect of survival of any animal species, but actual experience seems to show that immunity to ordinary infections is a far less potent factor in evolution than those studied by Darwin and his school. It would appear in the case of tetanus at least that susceptibility is acquired as the animal rises in the zoological scale as a secondary, though perhaps necessary, result of the possession of a nervous system of a certain degree of complexity. Secondly, the immunity due to a successful struggle against a disease is an acquired factor, and as such not transmitted, according, at least, to the majority of modern authorities. It might be objected that the capability of producing antitoxin might be a spontaneous feature, and one capable of being transmitted by heredity. This is probably true, but in this case it could only become a powerful agent in evolution if the disease were prevalent and an attack usual in the life-history of many of the individuals. This might occur in the case of tetanus, though here, as we have seen, the tendency in evolution is for the production of susceptibility rather than immunity; but it is quite impossible to see how the power of forming an antitoxin to el serum after the injection of that substance (to take one example of many which might be quoted) can be of advantage to any animal unless its habitat happens to be a pathological laboratory. Lastly, if the acquirement of immunity be of great importance in natural selection, we should expect those species to survive which de-

veloped natural, rather than the power to develop acquired, immunity, since the former would be always available, whilst the latter only come into action after the animal has successfully surmounted the hazard of an attack of the disease.

It appears more likely that the power to produce antibodies under certain circumstances is to be regarded as one of the essential properties of some forms of living protoplasm, and that its occasional value in the cure or prevention of disease is a mere accident. Thus the injection of eel serum leads to the production of antitoxin in all mammals, as far as is known, and this could only be of advantage to the animal if (1) eel serum happened to gain access to the tissues; (2) the animal recovered; and (3) eel serum again reached the tissues within a certain period. This is extremely unlikely to occur. Nor is natural immunity from poisons necessarily dependent on antitoxin; in fact, the common vegetable and other poisons do not lead to a production of antibodies, though if they did the possession of these substances would doubtless be of great value to animals in a wild state. If there is any advantage attaching to the power of forming antibodies, it is probably in regard to the solution of organized bodies, such as bacteria, or their sensitization previously to phagocytosis. It is quite conceivable that the advantage accruing from the possession of the power of forming antibodies has led to the selection of animals whose protoplasm has the property of developing an antibody to *any* foreign proteid, the useful side of this property being the formation of bacteriolysins and opsonins, the useless corollary being the production of antitoxins and precipitins.

The theory of passive antitoxic immunity does not present any difficulties of importance. It may be pointed out that it comes on as soon as the antitoxin reaches the blood-stream—*i.e.*, at once if the injection be intravenous, and after a delay of some duration if it be into the subcutaneous tissues or peritoneum.

Much attention has been paid to the question of the feasibility of administering antitoxin by the mouth and rectum. In general there is no doubt that this is useless, and under ordinary conditions it is not absorbed as such, being probably digested, and thus deprived of its peculiar characters. Under certain circumstances this appears not to be the case; thus it is held that absorption from the stomach may take place in young animals. Hence, perhaps, some of the undoubted benefit of the use of fresh raw

milk (in which the antibodies have not been destroyed by heat) in the feeding of infants. It seems also that absorption of antitoxins can take place in the intestine, and that the process may occur when gastric digestion is impaired or is prevented by artificial means; thus McClintock and King obtained successful results (in animals) in 93 per cent. of cases after the administration of a mixture of diphtheria antitoxin, opium, and of a saturated solution of salol in chloroform. It is possible that something of the same sort may occur in disease where the digestive powers are notably enfeebled, but this does not justify its exhibition in this way in cases of disease where every hour is of the utmost value. Recent researches have also shown that in some cases animals may be immunized per rectum; possibly success is only attained when the antitoxin goes sufficiently high up.

As regards the duration of the immunity conferred by a single dose of antitoxin, our knowledge is not very exact in the case of human beings. According to von Behring, Goodman, and others, an animal injected with diphtheria antitoxin retains its immunity for rather more than three weeks, and the duration is not markedly affected by the size of the dose given—for example, 20 units of antitoxin per kilogramme immunized a rabbit twenty-three days, 500 units twenty-six days. According to Goodman, the degree of immunity falls rapidly for the first two or three days, and then more slowly. According to him also, the immunity is greater than the amount of antitoxin in the blood would lead one to suppose, and it continues when antitoxin is no longer demonstrable in that situation. The duration of the immunity appears to depend upon the source of the antitoxin injected, being longer if the serum is homologous—*i.e.*, derived from an animal of the same species: thus von Behring showed that horses immunized by horse-serum antitoxin retain their passive immunity almost as long as if they had acquired active immunity from toxin injections, whereas the effect in rabbits, etc., as we have seen, is more transient.

But little need be added to what has been already mentioned incidentally with regard to the curative power of antitoxin. It acts, in the main, by neutralizing all toxin present in a free state in the blood or subsequently formed by the bacteria. In general, as has been pointed out, it does not repair the damage that the toxin has already done, nor remove the latter from its combination with the susceptible cells. It is perhaps hardly safe to assume that there is no trace of such an action, and it is by no means improb-

able that a very large excess of antitoxin present in the blood may have some slight power of attracting toxin from the tissues when the union is but recent. This, however, is quite unproved, but it is certain that cells which have been acted on by the toxin are not benefited by the subsequent action of the antidote.

It is hardly necessary to point out that antitoxin is devoid of bactericidal action, and that the removal of the infective agent is brought about by other mechanisms—in most cases, perhaps, by phagocytosis. Thus we shall see, in the case of diphtheria, that there is a rapid rise in the opsonic index about the time of the improvement in the local lesions; and we can hardly doubt, though direct evidence is lacking, that the preservation of the leucocytes from the deleterious action of the toxin when the latter is neutralized by means of antitoxin also plays its part in facilitating phagocytosis.

We now turn to the subject of natural immunity, an exceedingly difficult one, and one that is very far from being understood. What follows is for the most part extremely hypothetical.

It must be borne in mind that, though we speak of animals as susceptible or as immune to a given toxin, no hard-and-fast line can be drawn between the two conditions, and in some cases an unbroken series can be constructed between the most susceptible and the most resistant species. This is well seen in the case of tetanus. According to Knorr, the horse is the most susceptible animal to the toxin of this disease; the guinea-pig requires twice as much toxin per kilogramme of body-weight to constitute a lethal dose, the goat 4 times as much, the mouse 13 times, the rabbit 2,000 times, and the hen 200,000 times. Von Behring confirms these figures in general, but finds the mouse rather more susceptible than the horse. With the exception of the fowl these animals are all "susceptible." Of the "insusceptible" animals the hen is susceptible to large doses, but is also affected by much smaller ones if the injection be intra-cerebral. Other animals are still more resistant, such as the tortoise, lizard, cayman, larva of *Oryctes*, etc. The effect of other toxins on different species of animals has not yet been fully investigated.

It is necessary to realize that, before we can say that an animal is insusceptible or immune to the action of a given toxin, it is necessary for the animal to be kept, after injection, at a temperature at which this toxin can act. This subject has already been mentioned in connection with the functions of the haptophore and

toxophore groups. We have already seen reason to believe that the former can functionate (*i.e.*, the molecule of toxin can unite with the cell) at a low temperature, whereas the enzyme-like action of the toxophore radical can only act at the temperature of the human body, or one approximating thereto—this is in the case of tetanus. It is apparent, therefore, at the outset that there are two possible explanations for immunity to toxins: firstly, the toxin may find no cell with which it can unite—or, to use Ehrlich's terminology, no cell receptor having a combining affinity for the haptophore molecule of the toxin molecule; and, secondly, the union takes place, but the cell is not injured as a consequence—that is, the toxophore group is without action on the cell protoplasm.

1. Immunity due to an absence of suitable receptors.

This has been proved to exist in several cases. Most of these are due to the researches of Metchnikoff, who, however, does not express the fact in these words, but contents himself by saying that the toxin does not combine with the tissues. Thus, when the larva of *Oryctes* is injected with tetanus toxin, no symptoms develop, and if the blood of the animal be collected several months afterwards, it will be found to contain free toxin, as shown by the fact that it will tetanize susceptible animals. These experiments were carried out at a temperature of 30° to 36° C. Similar facts were observed in lizards;  $\frac{1}{50}$  c.c. of blood taken two months after the injection of tetanus toxin produced fatal tetanus in a mouse; the blood of a turtle (*Emys orbicularis*) contained toxin no less than four months after injection. In these cases the explanation of the immunity is clear, however we choose to express it: the toxin has no chemical affinity for the living protoplasm of any part of the animal, and is, therefore, powerless to injure it.

2. Immunity due to the insusceptibility of the cells to the action of the toxophore group.

Perhaps the best example of this is in the case, so often referred to, of the action of tetanus toxin on the cold frog, but other striking examples have been discovered by Metchnikoff. Thus, when the alligator is kept at ordinary temperatures (about 20° C.) and injected with tetanus toxin, that substance rapidly disappears from the blood, yet without the production of any tetanic symptoms, and without the appearance of antitoxin in the blood. If, however, the animal is kept at a temperature of 32° to 37° C., it produces antitoxin with great rapidity, though still without the

production of tetanus. Taking these two experiments together, we may regard them as constituting a definite proof of the existence of this form of immunity. The theory that the toxin disappears because it is anchored to the cells appears to be clearly demonstrated from the fact that antitoxin is produced, an occurrence which we could scarcely explain on any other hypothesis.

But it is hardly necessary to look for experimental proof of the occurrence of this form of immunity, since the fact that the blood of many species of animals is toxic for other species appears to constitute a ready-made demonstration of the fact. The most striking example is given by eel serum, a substance which is toxic for nearly all animals, except, of course, the eel itself. The serum of the horse is perhaps the least toxic of all sera, but even this has some poisonous properties. Whatever theory we may hold with regard to the mechanism by which the cells of an animal are nourished, it is hardly possible to avoid the conclusion that the proteid substances of the plasma must combine with the living protoplasm. And it is the proteids which make eel serum toxic to other animals. It appears, therefore, that the immunity of the eel to its own serum depends upon the fact that its protoplasm is not injured by the toxophore group, although the molecules of the toxin and protoplasm become united. (The toxicity of eel serum is dependent on a more complex structure than that of ordinary bacterial toxins, being, in fact, a cytolysin or cytotoxin, but this does not affect the argument.)

A consideration of the use of these poisonous sera to the animals which produce them may perhaps enable us to analyze the process a step further, and to attribute their immunity (*e.g.*, of eels to eel serum) to the power which their cells possess of using these "toxic" substances as sources of nourishment; and if this be so, we might perhaps apply this suggestion to explain the form of immunity to toxins under discussion. Thus, in the case of the alligator injected with tetanus toxin and kept in the cold, it is possible that the poison which combines with the cells is "digested" by them and used as nourishment. If this is so, we must assume that the molecule of toxin has, as far as its haptophore group is concerned, a close chemical affinity with the proteids normally present in the animal's blood and used by it in cell nutrition; and, further, that the toxophore group is not only innocuous to the cell, but is also no bar to the assimilation of the whole molecule by it. We have seen that in the case of the frog

the toxophore group only acts at a raised temperature. In the alligator we must assume that the elevation of the temperature has a similar but less marked effect ; it brings the toxophore group into a state of activity in which it is inassimilable by the molecule of cell protoplasm to which it is anchored, although the latter is not injured in any way. The result is, of course, the production of antitoxin, which approximates to the formation of the precipitins which occurs when non-poisonous foreign proteids are injected.

Thus it would appear that at the last analysis this form of immunity is in reality an expression of cell nutrition. A cell which can nourish itself on a given toxin is naturally immune to its action. It is hardly necessary, however, to say that there is no *direct* proof that any cell can extract nourishment from a bacterial toxin.

There is a third, and in some respects more interesting and important, form of immunity to toxins which is readily conceivable on theoretical grounds, and the occurrence of which is capable of experimental proof. We may define it as immunity due to the fact that some of the cells are insusceptible to the action of the toxin, but have a great combining affinity therewith, and thus shield from its action the susceptible cells, in which the combining affinity is less. We have already referred to the most striking and best known example, that of the immunity of the fowl to tetanus toxin injected subcutaneously as compared with its susceptibility when the injection is made direct into the brain. Some authorities have attempted to explain this on the assumption that there is a barrier *en route* which prevents the access of the toxin to the central nervous system. But if by this we are to imagine a physical barrier in the shape of a layer of endothelium or other tissue between the blood and the cells of the brain, the explanation is inadequate, since it would leave unexplained the nature of the immunity of this living barrier, nor would it explain the rapid disappearance of the toxin from the blood. The true explanation is certainly that the toxin combines rapidly with the cells of the body, or perhaps, as we shall see later, the leucocytes, and is thereby prevented from gaining access to the central nervous system. These relatively unimportant cells we must imagine to have the same nutritive relations to the molecules of toxin as have the cells of the heated alligator ; the two can unite, but the protoplasm is neither injured nor nourished. It seems probable that this



affords a sufficient explanation of the differences in susceptibility of the "susceptible" animals which figure in Knorr's list, and that the cells of the central nervous system in the warm-blooded vertebrates differ but little in their relation to tetanus toxin. Thus, in the case of the horse and the guinea-pig little toxin is bound to the body cells, and practically the whole amount makes its way to the brain wherever the injection is made. In the rabbit the body cells can absorb more, so that if only a small dose is given none may reach the more important and susceptible organs. The fowl marks the other end of the scale; the tissue cells must have an enormous avidity for toxin, and, unless absolutely gigantic doses are given, absorb the whole of it.

The objection may be raised that, the receptors of the body cells being of the same nature as antitoxin, the resulting combination of body-cell receptor and toxin should undergo dissociation, the poison being gradually passed on to the central nervous system, in the cells of which dissociation does not occur, since poisoning takes place; this refers to the case, *e.g.*, of the fowl. It is true that such a process appears to occur *in vitro*. If tetanus toxin be mixed with emulsions of many of the tissues, the two enter into a loose combination, so that if the fragments of toxin-charged tissues, after thorough washing in normal saline solution, are allowed to soak in that fluid, toxin is gradually liberated. It is only in the case of the central nervous system that stable non-dissociable compounds are formed. Several interpretations of the apparent anomaly might be suggested. It is, for instance, very probable that here, as in so many other phenomena in immunity, the real defensive cell is the leucocyte. Thus, Metchnikoff found that if he injected tetanus toxin into the fowl, and then, after an appropriate interval, excited an aseptic exudate composed largely of leucocytes, the fluid thus obtained would excite tetanus in a susceptible animal. Perhaps the chain of phenomena is as follows: The toxin first unites with the connective and other tissue cells, and so the amount that the leucocytes have to deal with at first is greatly diminished; dissociation takes place, and there is a steady stream of toxin from the tissues to the blood. This is dealt with by the leucocytes, which thus have time to increase in numbers and even in the insusceptible fowl the result of the injection is to cause a marked leucocytosis), and perhaps to become immune. It is useless to deny that these phenomena are difficult to harmonize with the side-chain theory as first expounded, or that Metchnikoff

has brought forward very strong (though not conclusive) evidence in support of his theory of the leucocytic origin of antitoxin; and, as we shall see, as far as our knowledge goes, we are led to believe that all antibodies are derived from the spleen, bone-marrow, and other organs rich in leucocytes.

The study of the affinity of bacterial toxins and of other poisons to various tissues and organs of the body is destined to play a part of great importance in our ideas of pathology and pharmacology. It has been especially investigated by Ehrlich, and in connection with immunity and susceptibility to toxins he recognizes four conditions which may occur:

1. In which no specific receptors occur in any part of the animal. This is the first form of natural immunity which we have discussed; the animal is absolutely immune, and cannot produce antitoxin.

2. Receptors are present, but only in tissues on which the poison does not act, or in tissues of little importance. Here the animal is immune, but antibodies may be produced. This is the case of the fowl *vis-à-vis* the tetanus toxin.

3. The receptors are distributed over various parts of the organism, and are present in the organs which are sensitive to the action of the poison. Here there is a relative immunity, and the degree of intoxication depends largely on the region into which the toxin is introduced. These animals may be immunized, though the process is one of some difficulty, owing to the sensitiveness to the toxin of vital organs, and, of course, antibodies may be produced.

4. The receptors are present only in vital organs which are sensitive to the poison. Here the animal is very susceptible, and immunization very difficult, involving the use of extremely minute doses or of toxoids. The action of tetanus toxin on the guinea-pig and horse provides examples.

## CHAPTER VII

### BACTERIOLYSIS AND ALLIED PHENOMENA

UP to the present we have dealt entirely with the mechanism by which the injurious effects of the infective bacteria are nullified. It is obvious that this is only one, though perhaps the most important, aspect of the question. We have now to study how the bacteria themselves are removed.

The bactericidal properties of the blood attracted attention very early in the history of bacteriology, and long before the beginnings of the science Hunter showed that blood had the power of resisting decomposition longer than other animal fluids. It was, however, the controversy which took place between Metchnikoff and the humoralist school which first focussed attention on this question, and led to the discovery of the *alexins* by Buchner, Nuttall, and others. The controversy is best discussed subsequently, and it is sufficient now to point out that it was found that the circulating blood had the power of killing certain bacteria, and that this property was even more marked in the serum. Thus, according to Lubarsch, 16,000 virulent bacilli will kill a rabbit if injected intravenously—*i.e.*, the blood has not the power of killing this number; yet 1 c.c. of fresh serum will destroy this number or more. It is obvious that the bactericidal substance or substances occur in the blood, and to a greater extent in the serum.

The properties of these bactericidal substances or alexins were investigated, and it was found that they were fragile bodies, readily destroyed by a moderate temperature ( $55^{\circ}$  C.), and that they disappeared spontaneously if the serum were kept for a few days. They were destroyed by acids and alkalis, and what was most important of all was that they were selective in their action—*i.e.*, those from a certain animal might be potent antiseptics as regards certain bacteria and inert towards others, whereas the serum of another species might have quite different actions. They appeared to be formed by the breaking down of leucocytes; hence their

appearance in the blood after clotting (when fibrin ferment is also liberated from these cells), and their absence from fluids containing none, such as the aqueous humour.

It was obvious that this was a foundation for a theory of immunity, but it soon became apparent that it did not explain the whole of the phenomena. An animal might be immune to a certain organism, yet its serum might not be bactericidal for it, or it might be susceptible and yet possess suitable alexins. Thus, rabbits are very susceptible to anthrax, yet have serum which kills the bacilli in large numbers, whilst the dog is much less susceptible, though its serum is very slightly bactericidal. Such facts prevented the alexic theory of immunity from making head-way until the discovery of Pfeiffer's phenomenon.

Pfeiffer found that when cholera vibrios were injected into the peritoneal cavity of a highly immunized guinea-pig they were not only killed, but dissolved, and this with great rapidity. The vibrios are first rendered immotile, and then lose their proper shape, becoming converted into spherical masses which stain badly. In a little while they become smaller, and disappear altogether, no trace being left.

Pfeiffer showed that the phenomenon could also be produced by injecting into the peritoneum of a normal guinea-pig a mixture of serum from an immunized animal and the culture of vibrios. This happened when an old specimen of serum in which the alexins had disappeared spontaneously was used, or a fresh specimen that had been heated to 60° C. Lastly, he found that if an old immune serum were injected into the peritoneal cavity and allowed to remain for a while, it regained its bactericidal powers, and could then dissolve the vibrios *in vitro* at the body temperature. The phenomenon was specific—*i.e.*, if serum from an animal immunized against cholera were used as described above, the vibrios of cholera were dissolved, but no others. If typhoid serum were used, typhoid bacilli showed degenerative changes, though they were not completely destroyed, but there was no effect on cholera vibrios.

The explanation which suggested itself to Pfeiffer was this: There is in the serum of highly immunized animals a substance which can exist either in an active or inert state. In the blood-serum or peritoneal fluid whilst in the body it occurs as an active substance, but it passes into the inert condition when kept for a few days, or very rapidly when heated to 60° C., and this inactive

substance can be rendered active again by contact with the living endothelial cells of the peritoneum.

The true explanation was given by Bordet, who showed that *two* substances are necessary for the phenomenon. The one is the inert thermostable substance which occurs in the serum of highly immunized animals, but not in normal animals, and which does not disappear on keeping. The second is a thermolabile substance which occurs in fresh serum, whether from a normal or from an immunized animal. This he showed by demonstrating that, whereas neither heated nor stale immune serum alone, nor fresh serum alone, had the power of leading to the production of Pfeiffer's reaction *in vitro*, this was caused when the two were used in combination. When a drop of fresh serum from a normal animal, some heated immune serum, and the cholera vibrios are mixed together and incubated, the whole train of phenomena was repeated.

The thermolabile substance taking part in the process was soon identified as the alexin of the earlier investigators, and the new substance was called by Bordet *substance sensibilatrice*. His theory was that alexin alone does not unite with the bacteria, unless these have been previously sensitized by the action of the substance in the immune serum. It was shown that the *substance sensibilatrice* has the power of uniting with the bacteria as follows: A culture exposed to the action of heated immune serum and washed by repeated centrifugalizations with normal saline solution is apparently unaltered, but the bacteria are dissolved by the action of normal serum, which has no action on unsensitized bacteria. Bordet supposed, therefore, that the immune serum alters the constitution of the bacteria in some way, so that the alexin can combine with them subsequently. He compared the process to the opening of a lock, which can only be effected by means of two keys, of which one (the sensibilatrice) must be turned before the other (alexin) can be introduced.

Bordet found that the essential facts could be reproduced exactly if red blood-corpuscles were substituted for bacteria. It was previously known that the serum of some animals possesses the power of liberating the hæmoglobin (hæmolysis) from the red corpuscles of other species, just as the serum of some animals can destroy certain bacteria. Bordet showed that the guinea-pig serum has normally no action on the red corpuscles of the rabbit, but that it becomes hæmolytic to the latter after a few injections

of rabbit's blood. Just as an animal which was formerly destitute of that power becomes able to dissolve the cholera vibrio after a few injections of that organism, so a guinea-pig, the serum of which had formerly no action on the rabbit's corpuscles, acquires the power of dissolving them as the result of an injection or two of rabbit's blood; hence, following the analogy with the cholera vibrio, the guinea-pig is said to be "immunized" to the rabbit's corpuscles, though there is here no question of the avoidance of any deleterious action. Further—and hence the importance of these discoveries in the theory of immunity—Bordet showed that the laws which govern bacteriolysis by means of immune sera were apparently identical with those governing Pfeiffer's phenomena. The fresh immune serum is hæmolytic; it loses its power on heating to 55° C., and it regains it on the addition of fresh normal serum. Further, the action is, to some extent at least, a specific one. An animal (A) injected with corpuscles of another species (B) will dissolve the corpuscles of that species, and may also have some action on those of animals closely allied zoologically. It is evident that bacteriolysis and hæmolysis are closely akin, and as researches on the latter phenomena are infinitely more easy than on the former, much work intended to elucidate the mechanism of immunity has been carried out on red corpuscles. This is to some extent regrettable, since the processes, though similar, are not identical, and it is unsafe to argue from one to the other without experimental verification.

It was this analogy between bacteriolysis and hæmolysis that led Ehrlich to an investigation of the latter phenomenon, and his researches led to a flood of new light being thrown upon the subject. Ehrlich introduced fresh names for the substances which Bordet had shown to be necessary for the phenomenon, and it will now be convenient to give a list of the various terms which the theories or caprices of various writers have applied to each.

The thermostable substance has been called :

Substance sensibilatrice, or simply sensibilatrice.	
Immune body.	Philocytase.
Amboceptor.	Immunisin.
Fixator.	Desmon.
Intermediary body.	Copula.
Interbody.	Preparator.

Whilst the thermolabile substance is spoken of as :

Alexin.	Complement.
Addiment.	Cytase.

We shall speak of the substances as amboceptor or immune body and as alexin or complement respectively. Objection may be



FIG. 24.—THE CONSTITUENTS OF FRESH IMMUNE SERUM, ON EHRLICH'S THEORY.

*a* = complement, *b* = amboceptor, *d* being its complementophile, and *c* its cytophile groups. Below, a red blood-corpuscle, showing its receptors.

This and the following figures are modified from Ehrlich, and are intended to illustrate his theories of hæmolysis. The black figures represent normal substances (corpuscles, complement), the white ones antibodies. It need hardly be said that they are absolutely diagrammatic.



FIG. 25.—THE IMMUNE SERUM SHOWN IN FIG. 24, AFTER HEATING.

The complement is destroyed, and amboceptor only remains.



FIG. 26.—NORMAL SERUM CONTAINING COMPLEMENT, BUT NO AMBOCEPTOR.

taken to Ehrlich's terms "amboceptor" and "complement," since, as we shall show, they imply a theory, but they are too firmly rooted to be displaced.

Ehrlich applied his side-chain theory to the study of the phenomena, and argued that amboceptor must be an antibody to the receptors of the red blood-corpuscle. If this is the case, it ought to unite with the corpuscles, and Ehrlich showed in the

following way that such actually occurred: He worked with the serum of a goat which had been injected with and was hæmolytic for the red blood-corpuscles of a sheep. He heated this immune serum to  $56^{\circ}$  C. to destroy complement, and added some sheep's corpuscles; the mixture was then centrifugalized, the supernatant fluid then pipetted off, and replaced by normal saline solution. The red corpuscles were to all appearance unaltered, but it was now found

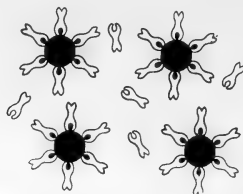


FIG. 27.—HEATED IMMUNE SERUM ADDED TO RED BLOOD-CORPUSCLES, WHICH ARE APPARENTLY UNALTERED, BUT ARE IN REALITY "SENSITIZED."

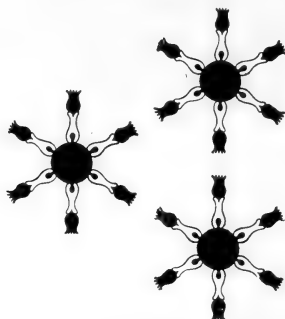


FIG. 28.—EFFECT OF ADDITION OF FRESH NORMAL SERUM TO SENSITIZED CORPUSCLES.

Complement is now linked up to the corpuscles, and hæmolysis takes place when they are incubated at  $37^{\circ}$  C.

that if a small amount of normal goat serum were added and the mixture incubated, hæmolysis occurred. It was evident, therefore, that the corpuscles underwent some change in virtue of their stay in the heated immune serum, though no alteration was obvious.

In a further experiment he showed that the change consisted in the abstraction of the amboceptor from the fluid. This appeared from the fact that if the supernatant fluid from the last experiment were pipetted off, fresh normal goat's serum added (to supply complement), and the mixture tested with sheep's corpuscles, no



hæmolysis occurred. Evidently it had been removed by the first addition of corpuscles.

Red corpuscles, therefore, have a combining affinity for amboceptor, and in further experiments Ehrlich showed that this combination takes place at ordinary temperatures or at low ones, down to  $0^{\circ}$  C. Expressed in the language of the side-chain theory, amboceptor has a haptophore group with a combining affinity for the receptors of the corpuscle, bacterium, etc., with which it

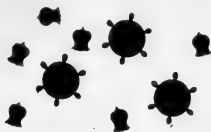


FIG. 29.—NORMAL GOAT SERUM (COMPLEMENT), PLUS SHEEP'S CORPUSCLES.

No combination. This is shown as follows: The mixture is centrifugalized, and to the corpuscles heated immune serum (amboceptor) was added (Fig. 30), whilst to the supernatant fluid corpuscles and heated serum were added (Fig. 31).

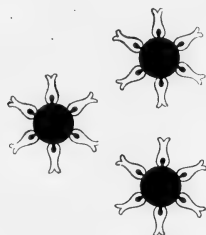


FIG. 30.—THE CORPUSCLES FROM THE PREVIOUS EXPERIMENT INCUBATED WITH HEATED IMMUNE SERUM.

No solution, showing that no complement had been abstracted in combination with them.

unites. We shall see reasons for believing that it may have a second haptophore group, and shall distinguish this first by the name of *cytophile haptophore*.

Ehrlich now investigated the behaviour of the second substance—the complement—with the red corpuscles by a precisely similar method, and found that the two had no power of entering into combination. Thus, normal goat's blood (containing complement) was added to sheep's corpuscles, and the mixture centrifugalized. To the corpuscles heated immune serum (amboceptor) was added, but there was no hæmolysis. Again, to the supernatant fluid sheep's corpuscles and heated serum were added, and hæmolysis

occurred; complement had evidently not been withdrawn from the fluid. Complement, therefore, will not unite with red corpuscles direct. It has no haptophore group with an affinity for the receptors of the latter.

This led Ehrlich to the theory that the complement united with the red corpuscle only indirectly by means of the amboceptor.

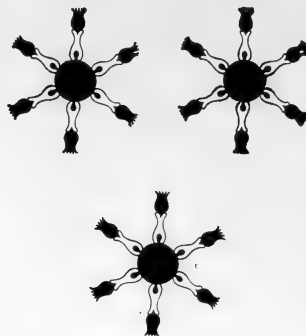


FIG. 31.—THE SUPERNATANT FLUID FROM FIG. 29, TESTED WITH HEATED IMMUNE SERUM AND SHEEP'S CORPUSCLES.

Solution takes place, showing that the complement had not been removed.

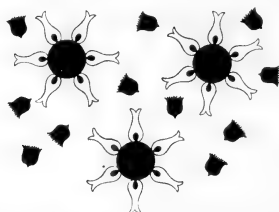


FIG. 32.—FRESH IMMUNE SERUM (OR A MIXTURE OF HEATED IMMUNE SERUM AND FRESH NORMAL SERUM) ADDED TO CORPUSCLES AT 0° C.

Amboceptor unites therewith, complement does not.

He pictured the latter as having two haptophore groups: a *cytophile*, which we have already mentioned, and a *complementophile*, with which the complement could unite after the former had seized on a receptor of the red corpuscle. The process of hæmolysis was supposed to take place as follows: In fresh immune serum or in a mixture of heated immune serum and fresh normal serum (*i.e.*, of amboceptor and complement) the two substances occur independently of one another. When the mixture is kept *in the cold*, and red corpuscles are added, the cytophile groups of the amboceptor molecules attach themselves to the receptors of

the red corpuscles, but the molecules of the complement still remain free, and do not attach themselves either directly or indirectly to the corpuscles. Nevertheless, there is a change in the combining affinities of the complementophile haptophore.<sup>1</sup> This is shown by the fact that if the mixture be raised to the body-heat, the molecules of complement attach themselves to these haptophores, exert a digestive or hæmolytic action through the amboceptor on the red corpuscles, and hæmolysis occurs. Hence, on Ehrlich's theory, the complement does not attack the cell or corpuscle direct (as Bordet holds, and as is implied in the term substance sensibilatrice for amboceptor), but it unites itself to one part of the molecule of amboceptor after another part of the latter has combined with the corpuscle. We will defer the discussion of these theories for the present.

For the sake of simplicity, we have assumed that complement will not unite with amboceptor until the latter has combined with its antigen. Ehrlich, however, assumes (on no very clear evidence) that the two substances may enter into a loose and easily dissociated chemical combination. This is hastened by heat and retarded by cold. The union between corpuscle and amboceptor is a firm one, which takes place at low temperatures, and which does not tend to dissociate, and after the combination amboceptor has a stronger affinity for complement, though even then a firm union only takes place at 30° C. or over.

Ehrlich explains the method of formation of amboceptor on the side-chain theory in this wise: He points out that for the absorption of substances of small molecule by the cell protoplasm the simple receptors, which, when cast off, constitute antitoxin, may suffice; but he says that when a giant proteid molecule (*e.g.*, of albumin) is anchored, it requires the action of a digestive ferment before it can be brought into a condition to be of use in the nourishment of the living cell. Hence it must be first broken down by means of a proteolytic enzyme, and it is of this nature that he imagines the complement to be. He holds, therefore, that the receptors or side-chains which are adapted to seize molecules of coagulable proteid possess *two* haptophore groups, the one to seize the nutrient material, and the other to seize a molecule of digestive enzyme from the surrounding blood or lymph. When the formation of such receptors is stimulated to

<sup>1</sup> Ehrlich does not make a definite statement as to this increase of affinity, but it seems a necessary deduction from the facts as he interprets them.

excess, and they break loose, they retain both their haptophore groups and constitute amboceptor. The stimulation and over-production is, of course, produced by the molecules of the substance injected (the specific antigens), be they red blood-corpuscles, bacteria, or, as we shall see, a whole host of other cells.

As a rough illustration of the nature of these seizing arms Ehrlich compares them with the tentacles of *Drosera*, which seize the nutrient material and then secrete a digestive fluid. The analogy is not exact, since the receptors do not form the digestive enzyme, but simply select it from without.

Ehrlich compares the action of the hæmolysins with that of the toxins, and points out that in each case there is a haptophore group adapted to seize on the red corpuscle to be dissolved or the cell to be poisoned, and an actively functional group with functions resembling those of an enzyme. He says that the hæmolysins are practically toxins in two parts, and that a combination of these parts is necessary before any action can be effected.

These discoveries and theories of Ehrlich led to a series of further researches, all of which were directly suggested by them, and the results of which appear to corroborate them to the full. Whatever the ultimate fate of the side-chain theory, there can be no doubt that it has led to an enormous increase of our knowledge on a most intricate subject. These researches have in many cases but little direct bearing at present on the subject of immunity, but they are far too important to be passed over without mention. We shall, therefore, summarize them as briefly as is possible when dealing with so complicated a subject. We will deal first with the explanation of the hæmolytic action which the serum of some normal animals has on the corpuscles of other species; for instance, normal goat serum will dissolve the red corpuscles of the guinea-pig. This was shown conclusively to depend upon the same mechanism of amboceptor and complement as in the artificial immune sera. The proof was as follows: Goat serum was mixed with guinea-pig's corpuscles, and the mixture kept at 0° C., and then centrifugalized. The theory would lead us to believe that the amboceptor had combined with the corpuscles, but that the complement had remained free. If this were so, the supernatant fluid would be found to have lost amboceptor. To test this a further addition of corpuscles was made, and the solvent power of the serum for these was found to be lowered. Evidently, then, sera which are naturally hæmo-

lytic to certain red corpuscles are so in virtue of containing amboceptors with cytophile haptophore groups which fit the receptors of these cells and complements which can dissolve them. He was also able to show, by an ingenious experiment, that when a normal serum can hæmolyze the corpuscles from different species of animals, it does so by the action of different amboceptors, and in some cases of different complements also. We must imagine, therefore, that normal serum contains numerous antibodies of the amboceptor type, and adapted to dissolve numerous foreign substances when they gain access to the blood; and it seems reasonable to believe that these normal antibodies and those which are formed as a result of the presence of the foreign substances play a part of the greatest importance in immunity.

The question then arose, Do animals possess or form hæmolysins adapted to the solution of their *own* corpuscles—in other words, an *autohæmolysin*? For instance, if a person sustains a large internal hæmorrhage, and the blood is absorbed, is he thereby stimulated to the production of a substance which dissolves his own red corpuscles? Ehrlich pointed out that such a phenomenon is unknown, and set out to investigate the reason of its non-occurrence. He selected goats as his experimental animals, and injected into them large amounts of mixed blood-corpuscles from other goats. In a week's time the serum was found to be powerfully hæmolytic, but only towards the corpuscles of other goats, never towards its own; thus the serum of the first animal experimented on was found to dissolve the corpuscles of goats 1, 2, 4, 5, 6, and 9 easily; those of 3 and 8 less powerfully; and those of 7 and of itself not at all. A second animal was treated just like the first, and it also developed a hæmolysin, but this did not act on its own corpuscles, nor on those of other goats in the same way as that of the first; evidently a different series of amboceptors had been formed. Ehrlich speaks of a hæmolysin formed by the injection of the corpuscles of a different species as *heterolysin*, that which acts on the blood of the same species as an *isolysin*; one which would act on the blood-corpuscles of the same animal would be called an *autolysin*, but this is never formed.

In attempting to discover the reason for this non-formation Ehrlich first proved that the amboceptor of the isolysin was anchored by the corpuscles which it dissolved, but not by those

of the goat from which it was derived. An obvious explanation would be that the latter did not possess any receptors which it would fit. Another suggestion might be that it had such receptors, but that they were already fully occupied by amboceptors occurring in the serum; but in this case they would attract complement, and solution would ensue. By a method<sup>1</sup> which will not be described here, since it would involve anticipation of facts not yet described, Ehrlich was able to prove the former view the correct one. The immunity, therefore, of the corpuscles of an animal to its own isolysin is due to a complete absence of suitable receptors in its corpuscles, and, we may add, in the entire organism.

He deduced, therefore, that each blood-corpuscle possesses numerous side-chains with haptophore groups, each of which, when injected into a living animal, is able to combine with a suitable receptor. If we take a particular variety of haptophore, which we will call  $\alpha$ , we can see that there are two possibilities after the injection; it may find no receptors  $\alpha$ , in which case there will be no antibody formed, or it may find such receptors. In the latter case there are also two possibilities: there may be receptors  $\alpha$  only, or there may be receptors  $\alpha$  and haptophore side-chains  $\alpha$ .

If there are only receptors  $\alpha$  and no side-chains  $\alpha$ , the injection of corpuscles with side-chains  $\alpha$ , will lead to an overproduction of receptors  $\alpha$ , and amboceptor will be produced. This will act as a hæmolysin to the corpuscles injected, but not to those of the animal itself, since they do not contain side-chains to which it can attach itself; it will be an isolysin, not an autolysin.

In the second possible case Ehrlich points out that the conditions for the production of an autolysin do occur, and such a substance might be produced and might do serious harm to the animal. But it would be produced at first only in small amounts, and the result might be that it would combine with receptor  $\alpha$ , which might then be stimulated and cast off and would form *anti-autolysin*.

<sup>1</sup> He first showed that the injection of an amboceptor from one animal into another of a different species would cause the production of an anti-amboceptor, and then showed that the injection of a serum containing isolysin into a goat whose corpuscles it dissolved produced anti-isolysin. These corpuscles contained receptors, since they fixed the isolysin. In the goat from which the isolysin came there was, of course, no production of anti-isolysin. Hence, he argued, it had no suitable receptors in its entire body.

There are, therefore, three possibilities : no formation of hæmolysin, the formation of an isolysin, and the formation of an anti-autolysin. In other words, if autolysins were ever developed they would be followed immediately by the production of their specific antibody.

But Ehrlich assumes—and our previous account of the iso-agglutinins leads us to be ready to accept his assumption unhesitatingly—that each corpuscle in every animal has numerous side-chains,  $\alpha$ ,  $\beta$ ,  $\gamma$ , etc. If such a corpuscle is injected into an animal of the same species,  $\alpha$  may lead to no result, finding no receptors ;  $\beta$  may lead to the production of an isolysin, and  $\gamma$  to that of an anti-autolysin. The existence of the latter substance could not, of course, be proved, since it was never possible to prepare autolysin.

Hence what Ehrlich calls the “pluralistic conception of the cellular immunity reaction.” There are, he says, in each bacterial cell numerous side-chains, to each of which an antibody is theoretically possible, and an ideal curative serum would contain all these antibodies. But in some cases, perhaps in most, only a few are found, and others cannot be developed ; for instance, in some animals it is impossible to produce anti-enzymes by the injection of enzymes. He explains this in two ways. The specific receptors may be of peculiar constitution, so that they cannot be cast off from the cell in the process of immunization : these he calls *sessile* receptors. Secondly, it is conceivable that the side-chains in question are normally produced by the animal cells, and that no antibody is produced to them, or, at any rate, that none accumulates.

But when either or both of the conditions arise, we may be able to get the antibodies from a second animal which we are unable to do from the first. Thus, if we imagine that the typhoid bacillus has twenty different sorts of side-chains, we may be able to get antibodies to some of them from the dog, to others from the rabbit, etc. Hence he suggests as an important principle in the formation of curative sera to use many animal species, mixing the sera of those which produce the antibodies required for use. (This, it must be pointed out, is not what is meant by a polyvalent serum, which is a serum formed by the injection of many strains of the same organism.)

Leaving this subject for the present, we will pass on to Ehrlich's researches into the nature of complements. Buchner, in his

studies on the alexins, assumed that the serum of each animal contained one alexin, though the alexins of different animal species were different. This view was also held by Bordet and others, and constitutes the "unitarian" view of alexin or complement. Metchnikoff holds that there are two alexins, or, as he calls them, cytases—macrocytase, which acts specially on red blood-corpuscles, cells, etc., and is joined by the large mononuclear cells; and microcytase, which acts on bacteria, and is formed by the polynuclear leucocytes.

As against these views, Ehrlich advances the theory of the multiplicity of the complements, the proof of which appears entirely satisfactory. According to him there are *numerous* complements in the serum of every animal, and these differ from one another in their haptophore groups, so that one can reactivate one ambo-

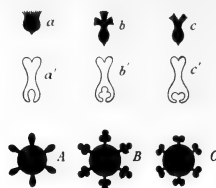


FIG. 33.—SHOWING SOME OF THE CONSTITUENTS WHICH CAN BE DEMONSTRATED IN THE SERUM OF A GOAT WHICH HAS BEEN INJECTED WITH SHEEP'S CORPUSCLES.

*a*, *b*, and *c* are the different complements, *a'*, *b'*, and *c'* the amboceptors which unite them to the corpuscles of the sheep (*A*), rabbit (*B*), and guinea-pig (*C*).

ceptor, another another. They differ in some cases in other respects. The first example which he was able to adduce in which there were at least two complements in a sample of serum was that of a goat which had been injected with sheep's corpuscles, and which dissolved those of the sheep, guinea-pig, and rabbit. But when this serum was heated to 56° C. for three-quarters of an hour, it was found to have no action on the corpuscles of the latter animals, whilst that of the former was unchanged. Evidently then, the complement which activated the amboceptor combining with the sheep's corpuscles was thermostable; that acting on the others, thermolabile. This was the first known example of a thermostable complement. In a later experiment he was able to prove that the complements taking part in the hæmolysis of rabbit's corpuscles and that taking part in the solution of guinea-pigs were different: the latter passes through a Pukall's filter, the former does not.



These methods of heating, filtration, etc., of course only separate the complements in certain lucky cases, and the more general method involves the removal of some of these bodies by means of sensitized red corpuscles. Thus fresh goat serum will reactivate heated normal goat serum in its action on rabbits and on guinea-pig's red corpuscles, the amboceptors for which are contained in untreated animals. It will also reactivate the heated serum of goats injected with rabbit's corpuscles, ox corpuscles, or dog's corpuscles. Ehrlich and Sachs found that if they added this fresh serum to rabbit's corpuscles, the complements which took part in the two latter reactions were absorbed, whilst the others were unaltered. Evidently, therefore, two complements at least are present in goat serum. When the serum was allowed to act on guinea-pig's corpuscles, the complements which reactivated the normal amboceptors for rabbit's and guinea-pig's corpuscles were removed, also that which activated the artificial ox amboceptor. By an elaboration of these methods Ehrlich and Sachs were able to demonstrate that these five hæmolytic actions depend upon five different complements, each perfectly distinguishable the one from the other. The subject will not be pursued farther, though there is an abundance of evidence pointing to the same end—that the serum of every animal contains a very large number of complements, some of which take part in one reaction, some in another. As a rule, complements which take part in hæmolysis have no action on bacteria, or but little, and this is all the truth in Metchnikoff's theory of macro- and microcytase.<sup>1</sup>

Ehrlich lays it down as a law that amboceptors from a certain species of animal are best complemented by complements from this species, and this is true in general; Muir has adduced some exceptions.

The main experimental basis for the unitarian theory was furnished by Bordet and Gengou. They found that if they added "sensitized" bacteria—*i.e.*, bacteria which had been placed in heated immune serum—in fresh serum, *all* the complements were removed, and the fluid would no longer dissolve sensitized red corpuscles, so that evidently the hæmolytic complement had been removed. Again, Bordet showed that if he added fresh serum to sensitized red corpuscles and allowed solution to take place, all complements, bacteriolytic as well as hæmolytic, were removed. These facts are not disputed, and we shall have occasion to refer

<sup>1</sup> See p. 286.

to them again, as they are of great importance. Their interpretation is, however, still uncertain, and they cannot outweigh the very precise demonstrations of numerous complements by Ehrlich and his school. According to them the receptor, which, when broken off, constitutes amboceptor, may have more than one complementophile group, each adapted to seize and utilize different complements. We must suppose that it can discharge its function if it is supplied with one of these complements; this is called the *dominant*. It may also seize other complements, so that all its affinities are supplied, and this is what takes place in Bordet's

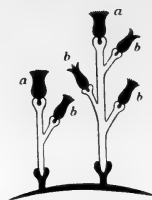


FIG. 34.—PLURICEPTORS ATTACHED TO A CORPUSCLE, AND SHOWING THE DOMINANT (*a*) AND NON-DOMINANT (*b*) COMPLEMENTS.



FIG. 35.—SOME OF THE CONSTITUENTS OF NORMAL GOAT SERUM.

*a*—Complement which dissolves sensitized sheep-corpuscles; *b*=that which dissolves rabbit's corpuscles; *c*=the normally occurring amboceptor for rabbit's corpuscles. The next two diagrams show how this is shown to be a biceptor.

and Gengou's phenomenon; these complements, which are not essential to the lytic action, are called *non-dominant* or subordinate complements.

Ehrlich and Sachs give an example of this. The amboceptor of normal goats for rabbit's cells and that obtained by injecting goats with ox corpuscles are, of course, both sensitized with normal goat serum. Now if we take rabbit's corpuscles and add them to fresh serum (amboceptor-complement), it is found that both the complements which might theoretically be present are removed, and the fluid will no longer reactivate heated immune serum for ox corpuscles. But if the action is allowed to go on for a short time only, and the corpuscles are then centrifugalized down and removed, it is found that only the non-dominant complement

is removed, and the supernatant fluid will still reactivate the action of heated immune serum on rabbit's corpuscles. This is not the invariable rule, and in other cases the non-dominant complements are not seized until the dominant has been anchored. In either case we can see an alternative explanation for the facts observed by Bordet and Gengou. Ehrlich points out that the presence of the power to bring these many ferments into relation with the giant molecule of the protoplasm must be of advantage in cell nutrition, as enabling the greatest possible effect to be produced, and quotes Hoffmeister as showing that the liver cell contains ten different ferments, and Delbrück as attributing five to the yeast cell.

The discovery that the hæmolytic action of snake venom depends on the mechanism of amboceptor and complement made



FIG. 36.—SHOWING EFFECT OF ADDITION OF RABBIT'S CORPUSCLES TO THE SERUM SHOWN IN FIG. 35.

The amboceptor unites with the corpuscle, and all the complements are withdrawn. The supernatant fluid loses its power of dissolving ox corpuscles previously sensitized. (See also Fig. 37.)

by Flexner and Noguchi, and the excellent subsequent work of Kyes, made us realize that the process might be even more complex. Flexner and Noguchi showed that snake venom contains two substances, one of which is thermostable, not being destroyed at  $90^{\circ}\text{C}$ ., and which in itself has no power of bringing about solution, and which is evidently equivalent to amboceptor. It may be reactivated by the other thermolabile substance or by the complements of normal serum. For example, horse corpuscles can be dissolved by heated venom and fresh ox serum, ox blood corpuscles by heated venom and guinea-pig serum, and so on. Kyes found that some corpuscles, washed from all trace of serum, could be dissolved by cobra venom alone; others required the concomitant presence of fresh serum of the same or other species. Thus the corpuscles of man, the dog, guinea-pig, etc., are dissolved by the venom alone, whilst those of the ox, sheep, and goat are not. He formed the theory (for reasons which will

not be discussed) that the solution of corpuscles *without* the action of serum was due to a complementing of the venom amboceptor by means of complements contained in the corpuscle itself, which he called *endo-complements*. This he proved experimentally. He argued that if he dissolved the red blood-corpuscles which contain endo-complements by means of distilled water, the solution should act as though it contained free complements, and should reactivate venom amboceptor, combined with corpuscles which it was unable to dissolve without the aid of serum. This was found to be the case. A laked solution of human corpuscles mixed with venom would hæmolyze the corpuscles of the ox, sheep, and goat; but a solution of ox corpuscles and venom would not dissolve the corpuscles of the goat or sheep. He showed that these endo-complements are thermolabile, though more resistant than most

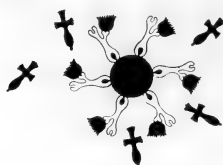


FIG. 37.—SHOWING EFFECT OF SHORT EXPOSURE OF RABBIT'S CORPUSCLES TO THE SERUM SHOWN IN FIG. 35.

The non-dominant complement is absorbed, and the supernatant fluid no longer dissolves sensitized ox corpuscles, whilst retaining its action on sensitized rabbit corpuscles. (In most cases the dominant complement is removed first.)

of those in sera. They are destroyed at  $62^{\circ}$  C. in half an hour. Kyes gave an additional demonstration of his theory by showing that if rabbit's corpuscles are soaked for twenty-four hours in normal saline solution, the endo-complement dissolved out, and could be demonstrated in the fluid, whilst the corpuscles were no longer hæmolyzed by snake venom without the addition of serum. He holds, therefore, that the corpuscles contain endo-complements able to dissolve them, but only when brought into organic relation with their protoplasm by means of amboceptor.

He next went on to demonstrate that a definite chemical substance—lecithin—can act as a complement to snake venom, and that either it or the serum-complement could act as a dominant so as to bring about solution, or that the two might act together. Lastly, he was able to prepare a combination of cobra venom and lecithin, which he calls cobra-lecithid, which is quite different in its physical and chemical characters from either of its

components, and which acts hæmolytically on all red corpuscles. It also acts much more quickly than ordinary solutions of venom even when activated with lecithin, and it is very thermostable, resisting boiling for six hours. It is evidently a definite chemical substance and of great theoretical interest, as being apparently an amboceptor-complement compound preparable in a pure state.

The analogy between the complements and the exotoxins leads to the inquiry whether the production of an anticomplement occurs when an active serum is injected into an animal of another species. Ehrlich and Bordet both proved this to be the case. He injected fresh horse serum into goats, and obtained a serum which would prevent the normal complementing action of horse serum.<sup>1</sup> He easily showed that this was not due to any action which it exerted on the red corpuscles (rabbit's) themselves or on the amboceptor.

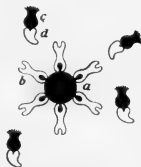


FIG. 38.—SHOWING THE EFFECT OF ADDING TO SENSITIZED RABBIT'S CORPUSCLES (*a, b*) A MIXTURE OF FRESH HORSE SERUM (CONTAINING COMPLEMENT, *c*), AND SERUM FROM A GOAT WHICH HAD BEEN INJECTED WITH HORSE SERUM AND CONTAINED ANTICOMPLEMENT, *d*.

The complement and anticomplement combine, and the corpuscles are unaffected; this is shown by the fact that they will dissolve on the addition of fresh complement.

It was clear, therefore, that it acted as an anticomplement. The question now arose, Was this action exerted on the haptophore or the zymophore group? The following experiment shows that it acts on the former: Sensitized red corpuscles were treated with a mixture of normal serum and of serum containing anticomplement: no solution took place. The corpuscles were then centrifugalized off and fresh serum added: hæmolysis occurred. Evidently the anticomplement had not acted on the zymophoric group, for if it had the haptophore group would have combined with the amboceptors of the sensitized corpuscles, and would have shielded them from further action when fresh serum was added. What

<sup>1</sup> There is a possible fallacy here, to be mentioned under our description of Bordet and Gengou's phenomenon. It may be simply a binding of the complement in the precipitate. This is discussed later, and at present Ehrlich's views will be set out as if they were definitely proved.

occurred was that the haptophore radical of the complement had been "blocked" by the anticomplement.

In a few cases anticomplements which will act on some only of the complements of a sera can be produced, and from the study of these additional evidence in favour of the multiplicity of complements has been adduced.

As regards the nature of these anticomplements, this is readily explicable on the side-chain theory. Ehrlich supposes that when a foreign serum is injected into an animal it may find no receptors for which it has an affinity; in this case it forms no anticomplement, and such cases are known to occur. Or it may find receptors which it can activate completely, just as the normal complement of the animal can do; in this case also there will (under ordinary circumstances) be no formation of anticomplement. Lastly, it may find receptors with which it can combine, but the resulting combination may be useless in the nutrition of the cell; in this

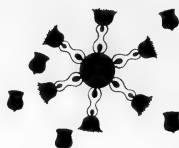


FIG. 39.—SHOWING EFFECT OF ADDITION TO SENSITIZED CORPUSCLES OF A MIXTURE OF COMPLEMENT AND COMPLEMENTOID.

The former unite with the complementophile haptophore groups of the amboceptor; the latter, which have decreased in combining affinity, do not.

case the receptor will be cast off, and will constitute anticomplement.

Lastly, Ehrlich was able to show that in some cases an *auto-anticomplement* may be formed—i.e., an antibody which can neutralize the complement normally present. The proof for this is elaborate, and will not be given here.

The change of toxins into toxoids is paralleled by the change of complements into *complementoids*. We have previously spoken of the complements as being destroyed by heat, but this is not quite correct; they lose their zymophore groups, but retain their haptophore portions, though these appear to lose some of their combining affinity. The proof of the existence of complementoids is simple: when injected into animals they call forth the production of anticomplements, just as toxoids call forth the production of antitoxin.

For some time it was found impossible to demonstrate the

presence of complementoids in test-tube experiments, and this was owing to the fact that the affinity of the haptophore group of the complements suffered loss during the process of heating, so that when a mixture of complement and complementoid was added to sensitized red corpuscles, the former combined just as well as if the latter were not present. "Blocking" of the complementophile group of the amboceptor appeared not to occur; but Ehrlich and Sachs were able subsequently to demonstrate such a phenomenon in this wise: Dog serum contains an amboceptor for guinea-pig's corpuscles, and this can be activated either by dog's or guinea-pig's serum. Now when a mixture of heated dog's serum, guinea-pig's corpuscles, and guinea-pig's serum was made, solution took place; but when the corpuscles and heated serum were added together, allowed to stand, and then centrifugalized and removed, no solution occurred on the addition of fresh

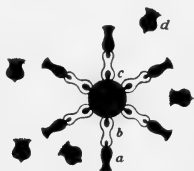


FIG. 40.—DEMONSTRATION OF "BLOCKING" OF COMPLEMENTOPHILE GROUPS BY COMPLEMENTOIDS.

$a$ =complementoid, and  $b$ =amboceptor in heated dog serum;  $c$ =rabbit's corpuscles (see text).

guinea-pig's serum. Ehrlich proved<sup>1</sup> that the combination of amboceptor and corpuscles took place as usual, and that it was then followed by a blocking of the free arms of these amboceptors by the complementoids present in the heated dog's blood. In this case, therefore, the complement had not suffered any appreciable change in combining capacity as a result of its conversion into complementoid.

It follows, therefore, that when we heat fresh serum, or allow it to stand a few days at the room temperature, we do not get rid of the complements altogether. This can be done, as shown by Von Dungern and others, by shaking the serum with cells (liver, kidney, etc.), yeast, fungi, certain bacteria, powdered charcoal, etc. This abstraction of complements by inert bodies is a non-specific process entirely unlike its fixation in hæmolytic, etc.,

<sup>1</sup> The proof is complete and conclusive, but too long to give here.

and Ehrlich compares it to a process of physical absorption. The presence of the phenomenon must be borne in mind, since it must make us careful how we interpret the mere disappearance of complement from a fluid after it has acted on articulate substances. The true test of specificity is its action on these substances, not its absorption by them. The yeast cells are in no ways injured through having absorbed complement.

We have not yet finished our study of the complements, but it will be convenient to continue it later, and to conclude here Ehrlich's studies on the antilynsins. Anticomplement has been already discussed, and we will now turn to anti-amboceptor.

This is a substance of nature different to any that we have previously studied, in that it is an antibody to an antibody. It is prepared by injecting an immune serum into an animal other than that from which it was derived. Thus, if an antityphoid (bacteriolytic) serum procured by immunizing a horse with typhoid bacilli be injected into a rabbit, the serum of the latter acquires the power of neutralizing the bacteriolytic action of the former. Antibodies to antibodies cannot always be produced; thus, anti-antitoxin is not yet known. Again, Metchnikoff was unable to prepare antispermotoxin by injecting a cytolytic serum for spermatozoa into the guinea-pig. In this case there can be no question of the absence of suitable receptors, for the animal's spermatozoa are attacked *in vitro* by the serum injected. Hence Ehrlich made the assumption of the presence of *sessile receptors*, which are not cast off when the suitable antibody is injected.

In studying the anti-amboceptor concerned in hæmolysis, Ehrlich first prepared a hæmolytic serum by injecting a rabbit with ox blood. When the serum of the latter was found to be powerfully hæmolytic, it was injected subcutaneously into a goat: 120 c.c. was given during an interval of two months. At the end of this time this goat serum was powerfully antihæmolytic. This was shown as follows: 0.00125 c.c. of (immune) rabbit serum was found to hæmolyze completely 1 c.c. of a 5 per cent. emulsion of ox corpuscles, provided that sufficient normal guinea-pig serum is added to provide complement. Now three times this amount (0.00375), plus 0.5 of the goat serum, was added to 1 c.c. of the emulsion of corpuscles, allowed to act at 40° C. for an hour, centrifugalized, and 0.15 c.c. of normal guinea-pig serum (complement) added to the corpuscular sediment. No solution took place, so that 0.5 c.c. of the anti-antiserum had completely neutralized three



dissolving doses of the antiserum. A control test showed that normal goat serum was without action.

Theoretically, two anti-amboceptors are possible: one which will combine with the cytophile group of the amboceptor, and one which will seize on the complementophile. In his earlier studies Ehrlich thought that the former was that actually produced when immune sera are injected into animals of another species. He later altered his opinion, and now holds that the substance actually formed is an antibody to the complementophile haptophore of the amboceptor. He was led to this conclusion by a discovery of Bordet's, which was adduced as evidence against the amboceptor theory, but which was ingeniously adapted to its defence by Ehrlich. The discovery was as follows: Normal rabbit serum when injected into a guinea-pig leads to the production of an anti-antibody, which neutralizes the hæmolsin formed by

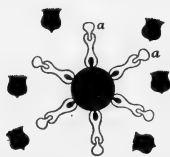


FIG. 41.—THE COMPLEMENTOPHILE HAPTOPHORE GROUPS OF AN AMBOCEPTOR "BLOCKED" BY ANTIAMBOCEPTOR (*a, a*).

immunizing with ox blood. This, of course, is readily explicable if normal rabbit serum contained an amboceptor for ox corpuscles, but this is not the case. Ehrlich argued as follows: An anti-amboceptor is obviously formed, but the normal rabbit serum injected contains no amboceptor—*i.e.*, no cytophile groups; hence the anti-amboceptors produced must be anticomplementophile. For this to happen it is necessary that this normal rabbit serum must contain complementophile groups, and this is obviously the case, since other amboceptors, each with a complementophile group, are present. Therefore, on the assumption that these groups are similar, even when they occur in different amboceptors, the case is clear. But are these complementophile groups similar? Does not this contradict Ehrlich's views on the multiplicity of complements? To avoid this difficulty, Ehrlich falls back on his polyeceptor theory. All or most amboceptors are really polyeceptors, differing only or mainly in their cytophile groups, and possessing numerous complementophile groups, which are similar or identical in different cases in the same species. But if this

is the case, anti-amboceptors should be non-specific; they are directed against the complementophile groups, which are the same in all amboceptors from the same species. This subject has not yet been fully investigated, but Pfeiffer and Friedberger have shown that the antibody to the immune body against the cholera vibrio also acts against the antityphoid serum. Ehrlich also showed that if anti-amboceptor be added to sensitized cells, these are protected against the subsequent addition of complement. This, of course, is attributable to the fact that the anti-amboceptor unites with the free complementophile groups, and "blocks" them against the subsequent access of the complement molecule.

Ehrlich leaves undecided the question as to whether an anticytophilic anti-amboceptor is ever produced, but holds that it might be formed if by any process we could destroy the cytophile haptophore of the amboceptor molecule. Under ordinary circumstances the complementophile group has a greater affinity for its cell receptor than has the cytophile for the receptors with which it could combine; union takes place between the former, and the latter is, so to speak, pulled out of the way of its affinity.

It is now advisable to recapitulate briefly some of the main points in Ehrlich's theory of the structure of the bacteriolytic and hæmolytic sera. Firstly, as regards complements: Of these there is very great number, and each is especially adapted for the solution of one or more varieties of cells, which it can dissolve in the presence of a suitable amboceptor; it is known as the dominant complement. Other complements, however, may help in the process, and these are termed non-dominant. In general terms the complements, which are especially active in hæmolysis, have but little action on bacteria, and *vice versa*. Secondly, as regards the amboceptor: This is really a polyceptor, and is so constituted that it can combine with the cell to be dissolved, on the one hand, and with a large number of molecules of complement, on the other.

Of the further complications of the theory which Ehrlich has introduced to explain new phenomena as they arise, of the amboceptoids, of loose and firm union, etc., we do not propose to speak. That the theory needs these complications in order to account for the phenomena is not necessarily in its disfavour, for the phenomena themselves are complex in the extreme. And it must be regarded as a strong argument in its favour that Ehrlich has again and again deduced results from this theory which subsequent research has shown to be correct; and very few hypo-

theses can be adduced which have been so rich in leading to the discovery of new facts.

It will now be necessary for us to glance briefly at some alternative hypotheses, and it must be pointed out that the comparatively short notice they will receive must not be taken to imply that there is less to be said in their favour. The trend of modern research tends on the whole against Ehrlich's views; yet in the history of immunity his researches will always be regarded as of the greatest interest and value.

It may be pointed out that the cast-off receptors of red corpuscles, bacteria, etc., may combine with the complementophile groups of an amboceptor, and thus appear to act as a cytophilic anti-amboceptor.

Bordet holds that the immune body is not an amboceptor at all—*i.e.*, that it does not act as a link between the corpuscle or bacterium and the complement, but that it sensitizes the former and renders it susceptible to the action of the latter. On Ehrlich's theory, amboceptor unites with cell and complement with amboceptor; on Bordet's, both substances unite with the cell direct. On the latter theory many, if not all, of the results observed by Ehrlich and others are explicable, and there appears to be no *experimentum crucis* by which the truth can be determined. A difficulty in the way of accepting Ehrlich's amboceptor theory is this: there is no proof that complement and immune body ever unite unless the latter has already combined with a cell, or with anti-amboceptor, or with free receptors. The only example to the contrary is supplied by Kyes' cobra-lecithid, which, though of great interest, can hardly be quoted as a case in point, since lecithin differs so markedly from the ordinary thermolabile complements of serum. Another case (in the deviation of the complements) in which this process *appears* to take place is extremely complicated, and the evidence in favour of the direct union of complement and antibody quite unsatisfactory. It follows, then, that we must either assume that the mere union of the cytophile group of the antibody with some other object increases the affinity of the complementophile group for complement (as has been done here), or we must agree with Bordet that cell and complement unite directly, but only after the latter has been prepared by the action of immune body. A very important observation of Muir's is of interest in this connection. Muir showed that after red corpuscles had been saturated with immune

body and then with complement, some of the immune body, but none of the complement, might be dissociated from the combination, and become free in the fluid. It is difficult, though perhaps not quite impossible, to reconcile this experiment with Ehrlich's theory, whilst it is really explicable on that of Bordet; but Muir (whose recent work on this difficult point should be consulted) has not come to a definite conclusion as to the nature of the combination.

Bordet also holds peculiar views on the subject of the union of corpuscles and immune body. According to Ehrlich, the combination is due to a strong chemical combining affinity between the two substances, and the resulting compound is a stable one, which is not readily dissociable. Cells thus activated possess a strong affinity for complement, and the whole process is a chemical one, dependent on ordinary chemical laws and obeying the laws of multiple proportions. This was, on the whole, confirmed by Muir, who proved that corpuscles combined with multiple doses of immune body took up multiple doses of complement; but he also showed that the corpuscle-antibody compound *did* dissociate, so that when corpuscles which had been saturated with immune body were placed in contact with normal corpuscles, some of the amboceptor left the sensitized and attached themselves to the normal. Bordet adduced evidence to show that the combination is of a remarkable nature, and dependent on obscure chemico-physical reactions. He took a sample of hæmolytic serum, and determined the amount of blood which it could hæmolyze when the two were mixed together. We may assume, on Ehrlich's theory, that all the suitable receptors in the corpuscles were saturated with amboceptor. But Bordet showed that if the corpuscles were added a little at a time, a very much smaller dose might take up *all* the immune body. Hence he compared the process to the staining of filter-paper when immersed in a dye. If the paper be added at once, it will be stained a uniform colour, whereas if it be added a little at a time, the first pieces will be stained deeply, the subsequent ones less and less, until the dye is completely absorbed. It would appear to explain the phenomenon equally well if we assumed that the corpuscles could be hæmolyzed by fewer amboceptors than they could take up, and there is some experimental evidence of this. Thus Muir showed that after red corpuscles have been dissolved by hæmolytic sera, the addition of more immune body will lead to the

absorption of more complement. It may be added, however, that many of the recent researches on antibodies point to them as being governed by the very complex laws, as yet not fully understood, which regulate the actions of colloids on one another and on crystalloids. This is discussed, though very briefly, in a subsequent chapter.

As regards the evolutionary significance of the facts of cytolysis (using the word to cover the solution of bacterial and all other cells), there are two theories which, though apparently widely different, have, on ultimate analysis, much in common. We have seen how Ehrlich explains the process of cell nutrition, the rôle which he attributes to complement, and the method in which he conceives the complex antibodies to be formed. On his theories, therefore, the fundamental process is one of cell nutrition, and we may imagine it to have become adapted to serve as a means of immunity during the course of natural selection: the animals which could make use of their mechanism of cell nutrition as a means of defence against invading micro-organisms would survive and perpetuate their species; the others could not.

We may add a few more considerations on this process of cell nutrition. We must assume that the food molecules of proteid which circulate in the blood are in an indifferent form, and are equally well adapted for the nourishment of cells of the brain, liver, etc., this method of transmission of nourishment having been found the most convenient and economical. The first requisite for the nutrition of the cell is that one of these molecules shall be brought into close relations with its protoplasm. Ehrlich speaks of this as being due to the selective action of the cell receptors, but we must not be misled by terms or diagrams, however convenient they may be to enable us to form a mental picture of the process. Ehrlich simply means that certain specialized molecules or groups of molecules have a chemical combining affinity for certain food molecules. This union is the first requisite, and in some cases it may be sufficient, and the molecule may be forthwith incorporated with the cell protoplasm. In most cases, however, this is not so, and the cell has to transform an indifferent molecule of proteid material into a form suitable for assimilation into itself. We now know that most, if not all, of the activities of a living cell in metabolism are discharged through the agency of enzyme action, and that each of the metabolic actions of a cell appears to depend on a special

enzyme. It is natural, therefore, that the cell should make use of soluble ferments in preparing the molecule which it has seized.

We now (thanks to the researches of Fischer, Hierfelder, and others) know that the action of these enzymes is strictly specific, and dependent on a stereo-chemical relation between the ferment and the body acted on, and that it is necessary in all cases for a union to be brought about between the two substances before the specific action is effected. Thus, if the two could unite in the blood, the molecules would be broken down *before* they reach the cells, and the food material would reach it in an indifferent form, which, however well adapted to some cells, might be useless to others. The substances, therefore, do not unite in the blood-stream at all, but the cell first seizes a molecule of food-stuff, and then one or more ferments which can change it into the exact form which the cell requires. Thus, the process of cell nutrition, according to Ehrlich, consists in the establishment of a link between the food and a suitable ferment after the former has been selected by the cell.

We may inquire why the cell does not form its own ferments. In certain cases it may do so; the existence of the endocomplements lends support to this view. But it is readily conceivable that it may be more economical in every way for the animal to limit this process of enzyme formation to certain specialized cells or tissues, just as pepsin is formed by the cells of the gastric glands. This is probably the case, and all experience goes to show that the cells to which this duty is delegated are the leucocytes.

Ehrlich, therefore, conceives of the cytotoxic mechanism of amboceptors and complements as being adapted, in the first place, to cell nutrition, and, secondarily, to the defence of the body by bringing about solution of foreign cells. Metchnikoff's views may be summarized as follows: He holds that *both* the substances taking part in cytolysis are ferments, and that both are adapted for intracellular digestion. His views on phagocytosis will be discussed subsequently, and here it will be sufficient to give the merest outline. He has shown that in the lowest Metazoa—*e.g.*, in the Coelenterata—digestion is an intracellular process entirely; the hypoblastic cells lining the alimentary canal seize food particles from the lumen and digest them, but form no secretion like those of the higher animals. In the cells which have seized and are digesting food particles in this way he was able to demonstrate

ferments allied to pepsin or trypsin, which had evidently been formed to digest the ingested food material. He then showed the importance of this process in the absorption of cells, etc., in the tissues of higher animals, and demonstrated that the two processes are altogether similar. Hence his views on the nature of the cytolytins arise easily and naturally. He holds that complement, or, as he calls it, *cytase*, is the digestive secretion of the leucocytes, and that, under ordinary circumstances, it is retained within the leucocytes; it is only set free when leucocytes are dissolved (phagolysis), either as the result of an injection of a foreign substance or in the process of clotting (this theory, as we shall show, is held by many other authorities). Thus in Pfeiffer's phenomenon the first result of the injection is phagolysis, and the ferments set free in the process immediately attack the cholera vibrios. Metchnikoff has proved clearly that the injection of almost anything into the peritoneal cavity leads to a diminution in the number of leucocytes, but the proof of the existence of phagolysis under these circumstances is less convincing. Cytase, therefore, is a digestive ferment adapted to deal with large masses of food substance rather than with molecules, as Ehrlich supposes, and is normally intracellular, being formed especially for intracellular digestion.

Metchnikoff sees in amboceptor, or *fixator*, a substance altogether analogous to enterokinase, and acting, like it, as an accessory digestive ferment, which has for its object the linking of the more potent ferment to the food to be digested. He regards it also as being formed in the leucocytes, and for this reason (amongst others, which will be enumerated subsequently): He states that the amount of fixator or amboceptor produced is proportional to the amount of phagocytosis which occurs during the absorption of the antigen. For example, he states that when defibrinated goose blood is injected into the guinea-pig subcutaneously there is but little phagocytosis, the blood being dissolved extracellularly, and but little immune body is produced; but when the injection is made into the peritoneum there is much phagocytosis and much development of antibody. There is certainly a remarkable difference between the various tissues as origins of antibodies, and, as a rule, the subcutaneous and connective tissues are most potent in this respect, the peritoneum next, and the circulating blood worst; but there is no sufficient evidence to show that this depends on the amount of phagocytosis

which occurs in these different situations. The site of the formation of an immune body will be referred to again.

Thus, whilst Ehrlich sees in the substances taking part in cytolysis evidence of the nutrition of the living molecules of cell protoplasm, Metchnikoff sees in them ferments which were also, in their first appearance in the animal economy, designed for the elaboration of the nourishment of the body or of certain cells therein, the difference being that, according to him, their primitive function was the digestion of large particles as well as of molecules. But in the highly-organized animals with which we have chiefly to deal this function has been changed, for in them phagocytes do not ingest bacteria and red corpuscles for the sake of the nourishment they contain, but to rid the body of invaders; the body as a whole is nourished through the agency of other extracellular digestive ferments, which are secreted into the alimentary canal. It follows, therefore, that cytase is unnecessary in the circulating blood, and, on this supposition, does not usually exist in that situation. Metchnikoff admits that immune body does so exist—and has indeed supplied a remarkable proof of the fact, since he showed that the spermatozoa of immunized guinea-pigs are combined with immune body, and only need the addition of fresh serum for cytolysis to occur. Cytase and fixator, on Metchnikoff's explanation of the phenomena, must be regarded as substances which in the evolution of the animal kingdom were first developed as digestive juices, but which now are entirely subservient to the defence of the body against invaders. According to Ehrlich, they are both in daily use in nourishment, and their defensive function is of less importance than their value in cell nutrition. In either case, the conception of immunity, as being fundamentally a process of nutrition, is a most striking one.

We have now to turn to a phenomenon which is of the highest theoretical importance, and which bids fair to be of great practical value in diagnosis. We have already referred to the fact that Bordet, the chief advocate of the unitarian theory of complement, showed that if red corpuscles be sensitized with amboceptor and added to fresh serum, the latter is deprived of *all* complementary activity. The same phenomena occur with sensitized bacteria. When, for instance, typhoid bacilli which had been acted on by heated typhoid serum were added to fresh blood, allowed to act, and then centrifugalized, it was found that the supernatant fluid had no longer the power of dissolving red corpuscles sensitized by suitable



amboceptor. These facts are admitted by Ehrlich, but he shows that they do not constitute any real evidence against the pluralist conception, since in some cases it may be shown that, though all complements are absorbed, this may be at different rates, and by stopping the process at a proper time, some may have disappeared, whilst others are left.

The practical importance of these observations arises from the fact that they give us a method by which we can demonstrate the presence or absence of an antibody to a given antigen, or of an antigen to a given antibody. For example, the sensibilatrice or amboceptor for the tubercle bacillus is very difficult to demonstrate, since the organism is so resistant that it is never obviously dissolved, even partially, in the most potent serum we can obtain. Nor are bactericidal experiments more promising, owing partly to the resisting power of the organism and partly to the technical difficulties. The only evidence (apart from the presence of agglutinins) which we have in favour of the formation of specific antibodies to tuberculosis is derived from an application of Bordet's phenomenon. The experiments were carried out as follows: A guinea-pig was injected with the bacillus of avian tuberculosis, to which it is but slightly sensitive, and the blood was examined by mixing it with an emulsion of the bacilli. If amboceptors were present they would combine with the bacilli, and draw to them all the complements of the fluid, which would thus lose its power of activating suitably sensitized red corpuscles. This was found to occur, and Bordet and Gengou deduced that the guinea-pig had formed antibodies to the slightly virulent tubercle bacilli. When, on the other hand, the guinea-pigs were injected with *virulent* human tubercle bacilli, no such antibodies could be demonstrated.

As an example of the recognition of an antigen by means of Bordet's phenomenon, we shall quote Bruck's demonstration of the presence of tuberculin, or at least of some derivative of the tubercle bacillus, in the blood of patients suffering from general tuberculosis. Here the problem is changed. We have a specimen of serum which we want to test, not for the antibody, but for the antigen. The procedure is as follows: The serum is heated and mixed with a serum known to contain antibodies to the tubercle bacillus (antituberculin of Höchst). To this mixture is added fresh guinea-pig's serum, and lastly sensitized red corpuscles. It is found that no hæmolysis takes place. In a control experiment, in which normal human blood took the place of that from the

patient, hæmolysis occurred; it followed, therefore, that the patient's blood contained derivatives of the tubercle bacillus, or (as we may fairly say) its toxins.

The technique of these experiments is somewhat difficult, but the results have been so important, and the method seems so likely to play a part of importance in clinical diagnosis, that it will be discussed in a further chapter.

Gengou's phenomenon is similar to Bordet's. It requires a little anticipation of facts to be discussed subsequently concerning the precipitins. These are antibodies obtained by the injection of proteid solutions, and having the power of uniting with these proteids to form insoluble precipitates. Gengou showed that in this combination of antigen and antibody the same absorption of complement occurred as in the case of sensitized red corpuscles or bacteria. The process is an extraordinarily delicate one, and it has been shown to be demonstrable with as little as 0.000001 c.c. of the antigen (in this case normal human serum), and may occur when the serum used is so dilute that no visible precipitation occurs.

It appears, further, that this process of fixation of complement is a general one, occurring whenever an antigen and its antibody unite, whether the antigen occurs in solid or liquid form, and whether the resulting compound forms a precipitate or remains in solution. It has been shown by Nicolle and by Armand-Delille to occur in the neutralization of tetanus and diphtheria toxins by their appropriate antitoxins, and by Pozerski in the interaction of papain and its anti ferment. It has been shown by Guedini that when hydatid fluid is mixed with the serum of an animal which has been injected therewith a similar phenomenon occurs, and this fact has been suggested and applied by Weinberg, Parvu, and Lanbry to the diagnosis of hydatid cysts in man.

The theoretical importance of this phenomenon arises from the light which it throws on the difficult subject of complementoids and anticomplements. We have seen that heated serum has no complementary activity, but that its injection into suitable animals appears to call forth the presence of anticomplements. A little consideration will show that this apparent anticomplementary action can be explained equally well by the absorption of the complements in a specific precipitate. We will take a particular case.

Goat serum heated to 56° C., and therefore containing no active

complement, is injected into a rabbit. This is supposed to develop an anticomplement in virtue of the complementoids it contains; we know that it also develops a precipitin which combines with the proteids of goat serum, and in doing so entangles any complement which may be present. Now the complement of goat serum can dissolve ox corpuscles when sensitized by a suitable amboceptor (*e.g.*, serum of a rabbit which has been injected with ox corpuscles). When, however, the serum of the normal goat is mixed with that of the rabbit which has been injected with rabbit serum and used in this way, no hæmolysis occurs. This Ehrlich explains on the supposition that the rabbit serum contains anticomplement, but it is also explicable, as Moreschi and Gay have shown, on the supposition that the complements are all absorbed in the precipitate (perhaps an invisible one) formed in the mixture.

It is obvious that either interpretation may be the correct one, or that both processes may come into play. Moreschi has investigated the process further, and his results tend to show that the experiment is best explained on the absorption of complement theory, and not by the presence of anticomplement. One of his experiments, and that very ingenious, will be described. He injected a rabbit with hen's egg albumin, and obtained a precipitin to that substance; this he found to act as an anticomplement to fowl serum, but not to that of the rabbit, guinea-pig, or goat, to which, of course, it was not a precipitin. He found, however, that if he added to any of these latter sera a minute trace ( $\frac{1}{100000}$  of its volume) of egg-albumin, it appeared to become so, the explanation being that a precipitate was formed, and that the complements were entangled in it.

The phenomenon has also been invoked to explain some extremely interesting researches of Pfeiffer and Friedberger, who thought they had demonstrated the presence of antibacteriolytic substances in normal serum. They prepared a mixture of cholera vibrios and normal serum, which they allowed to stand for some time, and then removed the bacteria by centrifugalization. This would remove any amboceptor which the serum might contain, and they thought that they could demonstrate that an anti-amboceptor still remained. They added to the serum thus prepared some anticholera serum in suitable amount and a lethal dose of cholera vibrios, and injected the whole into an animal, which invariably died; the amount of antiserum was sufficient to protect it if no

serum which had been exhausted by the action of cholera vibrios had been added. The anti-amboceptor which thus appeared to be present in normal serum seemed to be strictly specific; a serum which had been weakened by the action of cholera vibrios had no action on antityphoid serum, and *vice versa*.

Besredka attempted to explain these findings on the assumption that "free receptors" passed from the bacteria into the normal serum, and, when subsequently mixed with antiserum, combined with it, and so prevented its union with the living bacteria. They showed, in reply, that normal saline solution had no such action; but this is hardly conclusive, since receptors are dissolved from the bacteria much less powerfully in normal saline than in serum.

According to Sachs, who demonstrated a similar occurrence in hæmolysis, the inhibiting substance which actually occurs in the serum is an anticomplement. Gay, however, believes that the whole series of phenomena can be explained by the absorption of the complements in a specific precipitate. Thus, in Sach's experiment normal rabbit serum heated to 55° C. was treated with sheep's corpuscles. It was then removed, and added to a mixture of heated serum immune to sheep's corpuscles (obtained from a rabbit treated with sheep's corpuscles), fresh guinea-pig's serum, and sheep's corpuscles. No hæmolysis occurred, as it did in the control experiment, in which no "exhausted" normal serum had been added. His explanation was that the normal serum contained amboceptors which combined with sheep's corpuscles, leaving other amboceptors which have a greater affinity for complement than those combined with the corpuscles used as a test. Gay's explanation (which is almost certainly the correct one) is quite similar to Moreschi's explanation of the anti-complements. He believes that the immune serum contains also a precipitin to sheep's serum, and that in the "exhaustion" of the heated normal serum by the sheep's corpuscles some sheep's serum was added to it, the corpuscles in Sach's experiment not having been thoroughly washed. Thus a serum precipitate was formed, and the alexins or complements combined therewith, so that no hæmolysis occurred on the subsequent addition of sensitized corpuscles. He showed that if thoroughly washed corpuscles were used for the exhaustion the phenomenon did not occur, and gives other experimental proof.

Gay has also attempted to explain the process of deviation of the complements in bacteriolysis (the Neisser-Wechsberg pheno-

menon) in a similar way. The process is described at length on a subsequent page, but consists essentially in this: when bacteria are acted on by an *excess* of amboceptor, no bacteriolysis may occur, although sufficient complement is present. Gay's researches on this point have not been fully published at the time of writing, but he apparently thinks that soluble portions—receptors—may pass off from the bacteria into the fluid in which the latter are suspended, form a precipitate with the serum, and absorb the complement. Much evidence will be required before this can be established; it might perhaps explain the phenomenon *in vitro*, where the available amount of complement is limited, but the effect is also demonstrable in the peritoneum, where we should expect as much complement to be forthcoming as is required.

There is no doubt that the discovery of the absorption of the complements in serum precipitates has rendered uncertain many of the deductions in the process of hæmolysis which have emanated from the Ehrlich school, and the whole series of phenomena requires re-investigation in the light of the observations of Moreschi and Gay.

Experiments with bacteriolytic sera soon showed that, though they protected against specific infections, they only did so in certain doses. It is, of course, readily understandable that too small an amount might be without action, but it was also found that too large a dose might be equally inefficacious. Thus, Löffler and Abel obtained a serum which protected against *B. coli*, and found that when a lethal dose of this organism was given the animal was only protected when it had received between 0.02 and 0.25 c.c. of serum, larger as well as smaller doses being equally without effect. Similar results have been observed with sera against cholera, dysentery, malignant oedema, and other organisms, and seem to be general in dealing with bacteriolytic sera as opposed to antitoxin.

They can also be obtained *in vitro*, and since this was first shown by Neisser and Wechsberg, the phenomenon is often called after them. Their method was as follows: They worked with several organisms, amongst others with *V. Metchnikovi*, and with the serum of a rabbit which had been immunized against this organism, and had acquired strong bactericidal powers. Equal amounts of a broth culture of the vibrio were placed in a series of test-tubes, and to each a dose of heated immune serum was added. In all cases a uniform amount of normal rabbit serum

(complement) was added, and the mixture made up to constant volume with sterile normal saline solution. It was then incubated for three hours at 37° C., and finally 5 drops from each tube were plated out on agar and incubated. The result is shown on the table.

Amount of Broth Culture.	Heated Immune Serum.	Fresh Normal Serum.	Colonies Developing.
$\frac{1}{5000}$ c.c. in all cases.	1 c.c. 0.5 c.c. 0.25 c.c. 0.1 c.c. 0.05 c.c. 0.025 c.c. 0.01 c.c. 0.005 c.c. 0.0025 c.c. 0.001 c.c. 0.0005 c.c.	0.3 c.c. in all cases.	Infinity. Infinity. Many thousands. Several hundreds. About 100. About 50. None. None. About 100. Infinity. Infinity.

This shows that when  $\frac{1}{5000}$  c.c. of a one-day-old broth culture was treated with 3 c.c. of fresh normal serum (complement) and increasing doses of immune serum, there was no appreciable bactericidal action when more than 0.25 c.c. of the latter was used, or with less than 0.001 c.c. When the amount was between 0.1 and 0.025 c.c., or about 0.0025 c.c., there was appreciable action, and when it was between 0.01 and 0.005 c.c. it was complete.

The same authors also demonstrated a very remarkable phenomenon of exactly the same nature. A normal serum which has a certain amount of bactericidal action (owing to the presence of complement and of a small quantity of amboceptor) may have its action increased, diminished, or nullified by the addition of a powerful immune serum. This is shown by the following table, which demonstrates the action of normal guinea-pig serum on *V. Nordhafen* by itself and after the addition of variable doses of heated immune serum.

This table shows that there is a definite relation between the amounts of amboceptor and of complement which have to be present to produce a maximal effect. In the case of the normal serum, it is evident that there is more complement than can be used by the amount of amboceptor present, and that the bactericidal action of the mixture increases as immune serum is added. 0.05 c.c. of normal serum was without obvious bactericidal effect on  $\frac{1}{100}$  c.c. of the culture, whereas the same amount plus 0.01 c.c. of

heated immune serum destroyed a very large number of the bacteria. When, however, the immune serum is added in larger proportion than this it does harm instead of good: 0.5 c.c. of

Amount of Culture.	Amount of Normal Serum.	Number of Colonies after addition of Variable Amounts of Heated Immune Serum.			
		No Serum added.	1 c.c. added.	0.1 c.c. added.	0.01 c.c. added.
$\frac{1}{100}$ c.c. in all cases.	1 c.c.	None.	Many thousands.	A few.	None.
	0.5 c.c.	None.	Almost infinity.	About 100.	None.
	0.25 c.c.	A few.	Infinity.	Several hundreds.	A few.
	0.1 c.c.	Several thousands.	Infinity.	Infinity.	About 100.
	0.05 c.c.	Infinity.	Infinity.	Infinity.	Many hundreds.
	0.025 c.c.	Infinity.	Infinity.	Infinity.	Infinity.

fresh serum completely sterilizes the amount of the culture used, whereas after the addition of 1 c.c. of immune serum its action is barely noticeable. An excess of amboceptor shields the bacteria from the solvent action of the complements. These test-tube experiments explain the results obtained *in vivo*, and enable us to form some idea as to the mechanism of the process.

Neisser and Wechsberg offered the following explanation: They assume that the molecules of complement and amboceptor unite in the mixture before the latter has attached itself to the bacteria. Owing to the excess of amboceptor, there will not be sufficient molecules of complement to go round, and it will follow that a variable proportion of molecules of amboceptor will be uncomplemented. Unless we suppose that the union of the complement has changed the affinity of the cytophile group of the amboceptor for the bacterium, it will follow that not all the amboceptors which attach themselves to the bacteria will be charged with complement, and therefore able to exert a bacteriolytic or solvent action. To take a concrete case, let us suppose that there are twice as many molecules of amboceptor as of complement. Half the molecules of the former will be complemented, and it will follow that, though all the (appropriate) receptors of the bacterium are occupied by amboceptor, only half of these will have their complementophile groups occupied, and this may not be enough to injure it.

They also imagine the possibility of the combination altering the affinity of the cytophile group of the amboceptor for the cell. It may *diminish* it, in which case fewer complemented molecules

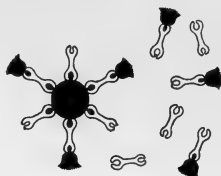


FIG. 42.—FIRST POSSIBILITY.

The affinity of the amboceptor for complement is unaltered, as a result of the union of the former with a bacterium.

of amboceptor would attack the bacterium than ever, and the phenomenon of deviation of the complements would be even more marked. The union might also (conceivably) *increase* this affinity; in this case the amboceptors which were complemented would

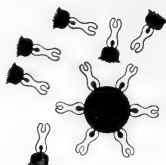


FIG. 43.—SECOND POSSIBILITY.

The affinity of the amboceptor for complement is diminished, as a result of the union of the former with a bacterium.

seize on the bacterial receptors, to the exclusion of those which were not, and the phenomenon of deviation of the complements would not occur. This Neisser and Wechsberg think might occur in the case of hæmolysis, for in this process deviation has not

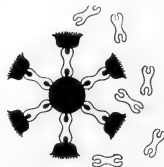


FIG. 44.—THIRD POSSIBILITY.

The affinity of the amboceptor for complement is increased.

been demonstrated, and solution occurs even when there is a great excess of amboceptor. In the case of hæmolysis by snake venom, however, Myers and Stephens showed that the process might only take place when medium doses of venom were added,



amounts too large or too small being without effect. This was also corroborated by Kyes and Sachs, who showed that in presence of an excess of venom (amboceptor) the addition of a hæmolyzing amount of complement might be without effect. Now Kyes had already shown that venom *does* unite directly with one of the substances (lecithin) which can complement it, and this appeared strong evidence in favour of Neisser and Wechsberg's explanation. But Noguchi afterwards showed that the protective action of large doses of venom protects the red corpuscles against the action of hæmolytic agents other than complements. Corpuscles so treated are not dissolved by distilled water or by tetanolysin. It appears, therefore, that strong solutions of venom have a curious hardening effect on red corpuscles, rendering them resistant to hæmolytic agents of all sorts. This effect is apparently entirely different from the protection of a bacterium by excessive doses of amboceptor, and should not be used as evidence to support Neisser and Wechsberg's explanation.

Morgenroth has attempted to explain the absence of the deviation of the complements in hæmolysis by alleging that in this case the amboceptors and complements do not unite until the former have united with the red corpuscles; this, of course, is the point at issue. He finds that by adding anti-amboceptor (for the cytophile group) to a mixture of amboceptor and complement the phenomenon of deviation can be reproduced. But his experiment can probably be explained by means of Gay's observations, to be explained subsequently, and in any case his anti-amboceptor would only act as a free receptor of a red corpuscle, and the amboceptor attached to it would be in the same condition as one anchored to a corpuscle, which we know can absorb complement. In any case, his experiment proves little or nothing.

Some experiments by Meakins afford the best evidence in favour of the alteration in combining affinity undergone by amboceptor after union with complement, and call for short reference, though their interpretation is somewhat uncertain. Meakins experimented with corpuscles which had been very thoroughly washed so as to remove all trace of serum. The importance of this precaution will appear subsequently. He found that when he added a large dose of heated immune serum and a small dose of normal serum (complement) to corpuscles thus washed, and allowed them to act, no hæmolysis occurred. The corpuscles, however, were sensitized; for when they were centrifuged down,

washed, and treated with more normal serum, hæmolysis took place. It thus appears that, in the first instance, the corpuscles took up only those amboceptors which had no attached complement. It would appear, therefore, that amboceptor may unite with complement whilst free, and that when it has done so its affinity for the corpuscle is diminished. This is one of the conditions which Neisser and Wechsberg imagined, and which they showed would make the phenomenon of deviation even more marked. And Meakins shows, though not in a very clear way, that it does actually take place with corpuscles that are well washed. The reason why it does not take place under ordinary circumstances is explicable on the basis of Gengou's reaction.

But this theory cannot be regarded as entirely satisfactory. It assumes, in the first place, a direct union between complement and amboceptor before the latter has united with the cell or bacterium, and of this there is no definite evidence apart from this phenomenon. Ehrlich, it is true, supposes a feeble union between the two which easily dissociates, even at low temperatures, so that a cell which has been placed in contact with a mixture of the two at  $0^{\circ}$  will become saturated with amboceptor devoid of complement. But this appears to be a fatal objection to Neisser and Wechsberg's explanation; for if the hypothetical amboceptor-complement combination dissociates readily at a low temperature, it should do so still more at a high one. Now the receptor-amboceptor-complement will not dissociate, or only to a very slight extent, since lytic action will at once commence. It follows that the amboceptor-complement molecules will gradually all dissociate, and the complements will sooner or later find their way to the anchored amboceptors, and there remain. The process may be delayed, but after a time all the attached amboceptors will become complemented. And if, as seems implied in Ehrlich's writings, the affinity of the complementophile group becomes raised in virtue of the union of the cytophile group, with its appropriate receptor, this process will take place all the more quickly.

It appears much more likely that the Neisser-Wechsberg phenomenon is an example of a reaction of a kind which has already attracted much attention, and which may possibly modify fundamentally our views on the antibodies, toxins, etc. They will be referred to again when we deal with the agglutinins, and it is sufficient to say here that in other cases, when there is no question

of two bodies, and therefore of the validity of Neisser and Wechsberg's explanation, antibodies and other substances have their action diminished or suspended when they are present in excess; thus with a great excess of agglutinin there may be no agglutination. And Detre and Sellei have shown that phenomena which are apparently somewhat similar occur in the hæmolysis induced by mercury perchloride.

In view of the important rôle in immunity which must be ascribed to alexin—and, as we shall see, it acts not only as a bacteriolytic agent, but also plays a part of great importance in phagocytosis—an inquiry into its source becomes of paramount importance. The theory was put forward very early in the history of the subject, before the mode of action of alexin and its dependence on immune body were understood, that it was derived from the leucocytes, either by a process of secretion or disintegration. This was first suggested by Hankin, who prepared a substance having bacteriolytic powers from extracts of the lymphatic glands and spleen; this, however, was probably not a true alexin. Experimental evidence of a more convincing kind soon followed; thus Denys, Van de Velde, and others, caused the production of aseptic exudates rich in leucocytes by injecting various irritants into the pleural cavity of animals, and found that the more leucocytes present the greater the bactericidal action of the fluid. Buchner performed similar experiments, using aleuron emulsions to produce the exudate, and found that the richly cellular material had a greater bactericidal effect than blood or serum; in his experiments he avoided the possibility of phagocytosis (which probably explained some of the results of other investigators) by freezing the fluids before determining their potency. A large number of other researches were made about this time, and all pointed in the same direction, and in the early nineties it was fairly generally held that the alexins were given off in some way or another by the leucocytes. This view was strongly held by Metchnikoff, whose microcytase must be regarded as identical with the alexin or complement which acts on the bacteria; and this microcytase he holds to be produced by the polynuclear leucocytes under certain circumstances, which will be discussed subsequently.

With the discovery of the compound nature of the bactericidal substances the question entered on a new phase, and new methods of investigation were seen to be necessary. Since then very

many researches have appeared, the point at issue being whether the alexin or complement is formed from the polynuclear leucocytes (for there is no evidence pointing to the lymphocytes). Some of the most important of these must be briefly summarized.

1. Various observers have investigated the relation between the number of leucocytes in a given fluid and the amount of complementary action. Thus Bulloch, working with hæmo-complement, found its amount closely proportionate to the number of polynuclear leucocytes. His figures are very convincing, and the only alternative explanation of his results is that the injection of any substance which stimulates leucocytosis stimulates at the same time the hypothetical complement-producing organ or tissue. Similar observations have been made under natural conditions by Longcope, who found that in disease in man the occurrence of hyperleucocytosis is accompanied by a rise in the amount of complement. On the other hand, Guseff's investigations on similar lines were entirely negative, and he was unable to trace any parallelism between the two; and Briscoe, dealing with peritoneal fluids, arrived at a similar result. These results may be taken as typical of the whole. A series of experiments by an able worker is apparently conclusive in one direction, and appears to establish the point beyond controversy, but is immediately met with another on similar lines, pointing to a diametrically opposite result.

Other investigations on similar lines are those of Bordet, who found that the œdema fluid poured out in passive congestion in an immunized animal—a fluid poor in cells—contains immune body, but no complement, and that the aqueous humour is entirely devoid of both substances. This latter fact has been corroborated by Levaditi, who adds that when, after tapping, the aqueous humour becomes rich in leucocytes, it acquires alexin at the same time.

2. Many observers have attempted to prepare complement from emulsions of polynuclear leucocytes, from bone-marrow, or from lymphoid organs. The results have been diverse in the extreme, and this diversity appears due in part to the fact that substances other than true alexins or complement may cause, or play some part in the causation of, both hæmolysis and bacteriolysis. In particular, we may refer to the important rôle of *lecithin* and its allies in hæmolysis, and possibly in the destruction of bacteria also. It is quite possible, for instance, that the

hæmolytic complement prepared by Levaditi from autolyzed lymphoid tissue was one of these lipoid substances; it was soluble in alcohol and thermostable. The substance prepared by Morgenroth and Korschun from extracts of various organs was probably similar in nature, since they showed it to be thermostable, and not to give rise to the production of antibodies on injection. It may be at once admitted, as pointed out by Conradi, that autolytic changes occurring in various organs give rise to the production of bactericidal substances; these may, perhaps, be organic acids or other simple substances, but they do not help us in the inquiry as to whether complement (having the power to dissolve sensitized bacteria or red corpuscles) is formed from the leucocytes.

The most important researches on this point are those of Schattenfroh and Petrie, and they are mutually contradictory. Schattenfroh washed the leucocytes thoroughly in saline solution, added it to the heated serum, and obtained a bactericidal effect. His results were corroborated by Lastchenko. Petrie's researches were carried out with great care, and he was especially particular to remove every trace of complement adhering to the leucocytes by repeated washing in normal saline solution. (Ascher had previously shown that some trace might remain when the process had only been carried out three times.) The leucocytes were obtained from aleuron exudates, and after washing they were frozen to the temperature of liquid air, and ground to an impalpable powder, the method used being that of Macfadyen for the preparation of endotoxin. His results were entirely negative, and he failed entirely to reactivate heated bactericidal serum by any of his extracts. Lambotte failed equally, working on lines more like those of Schattenfroh.

Petrie's experiments seem conclusive on the point which he investigated, and it seems quite certain that polynuclear leucocytes do not contain alexin or complement *as such*; they do not, however, negative the possibility that they may contain a precursor of this substance, which may either be secreted or set free during the *natural* solution of the leucocyte, in either case being converted into an active form during the process. Both these views have been widely held, and we shall discuss them later.

3. In the process of coagulation of the blood large numbers of the leucocytes, and especially the polynuclears, are disintegrated and partially dissolved, and these disintegrated leucocytes are

generally accepted as the source of the fibrin ferment. If the complements are also set free during the solution of the leucocytes, there should be far more present in the serum than in the circulating plasma; it might happen, indeed, that the plasma might be devoid of any complementary action, though this does not necessarily follow.

The difficulty of investigating this subject is very great, and the evidence is mostly indirect. As far as it goes, it seems to point to the fact that the plasma is alexin-free, or, at any rate, poorer than the serum. The main *direct* researches into this subject are those of Gengou and Falloise, and here, again, they point in a diametrically opposite direction. Gengou (whose results have been accepted in their entirety by Metchnikoff) worked with plasma obtained by preventing coagulation by collecting the blood in paraffined tubes and centrifugalizing forthwith—a method certainly less open to objections than any dependent on the addition of anticoagulants, but difficult in execution. He found that plasma thus prepared was devoid of bactericidal action, though the serum from the same animal possessed it. In some cases the plasma betrayed some action, perhaps because a partial coagulation had taken place, but it was always less potent than the serum. These results have been opposed by numerous investigators, using various methods of inhibiting coagulation. Thus, Falloise used intravenous injections of peptone, the addition of sodium oxalate or fluoride; Petterson used oxalate and citrate, and Hahn histon, and in all cases no appreciable difference in alexin content between the plasma and serum was found. But this is hardly a fair test. Peptone is generally believed to be a leucotoxic or leucolytic agent; and in the case of the citrated or oxalated blood it is obvious that (if leucocytes are the origin of fibrin ferment) some leucolysis has occurred, since the cell-free fluid coagulates on the addition of calcium. Falloise also worked with paraffin tubes on Gengou's method, and found that the plasma contained as much hæmolytic complement as the serum. Lastly, both he and Lambotte worked with plasma obtained by centrifugalizing (or by allowing sedimentation to occur in) the blood in a vein isolated between two ligatures and kept at a low temperature, and with the same results. Delezenne, however, criticizes these results by pointing out that plasma obtained in this way coagulates instantly in glass tubes at the ordinary temperature, a phenomenon indicating that fibrin ferment is present, and that leucocytes have

become disintegrated, or have at least performed an act of secretion which they do not perform in the living blood. The conditions, therefore, are not altogether natural.

We are forced to rely to a large extent on indirect evidence, and this, as far as it goes, is strongly in favour of Gengou's view.

4. Ainley Walker's experiments dealt with the amount of complement present in the successive amounts of serum squeezed out from a clot, and showed that this gradually rises, the first few drops containing but little, and the quantity gradually increasing until it attains its maximum in about twenty-four hours. Other explanations are possible, but it is at least probable that the cause of this increase is the destruction of the leucocytes which is known to occur during the process of coagulation, and that the formation of complement is quite analogous with that of fibrin ferment. The serum first formed is weak in complement, the destruction of the leucocytes having only just commenced; and it seems a fair deduction that, if we could examine the plasma when no leucolysis has occurred, we should find that it contains much less, or none at all.

This view is supported by Levaditi in a series of researches on the fate of cholera vibrios injected into the circulatory system of immunized guinea-pigs, care being taken to avoid as far as possible the destruction of leucocytes. Under these circumstances he finds that the Pfeiffer phenomenon does not take place, or does so much more slowly; the vibrios circulate in the blood for half an hour or more without showing the least trace of granular transformation, and are rapidly taken up by the leucocytes. In a similar series of experiments on hæmolysis he was able to prove the existence of sensitized red corpuscles in the circulation, although the animal did not present the least trace of hæmoglobinuria, and was of opinion that this was due to the absence of free alexin in the plasma. The anæmia due to the injection of hæmolytic amboceptor he believes to be brought about by the destruction of the red corpuscles within the macrophages of the spleen.

As a third item of indirect evidence we may quote the experiments of Lubarsch and others, to the effect that an animal may be killed by the intravenous injection of a smaller number of bacteria (*e.g.*, of anthrax) than are destroyed by a small quantity (1 c.c. or less) of serum. The simplest explanation is that the blood contains an abundance of immune body, and the failure of the defensive

mechanism is due to lack of complement. It would, however, be unwise to attach too much importance to this proof.

The difficulties in coming to a conclusion on this subject are great, but on the whole it would appear that the evidence is somewhat in favour of the views of Metchnikoff, and that in all probability there is no free alexin, or but little, in the plasma, and that the main source of this substance is the polynuclear leucocyte, but we cannot regard this as definitely proved. This being so, the discussion as to whether the formation of alexin is a vital secretory process or a phenomenon of cell death and solution cannot be regarded as of great importance. Here again the experimental work is most inconclusive, Lastchenko holding that living leucocytes give off alexin when suspended in heated serum, whilst Lazar found that it is only set free when some of the leucocytes are destroyed. Kanthack's experiments may perhaps be quoted at this point. He found that anthrax bacilli immersed in frog's lymph become surrounded by eosinophile cells. After a time these cells discharge their granules, and the bacilli soon begin to show signs of injury, becoming less refractile and losing their sharp-cut outline. After this these leucocytes move away from the bacilli, from which we might argue that they are uninjured, and that the solution of their granules is a vital action, and exactly equivalent to the secretion of pepsin by the gastric cells. This, of course, does not exclude the possibility that alexin might also be liberated during the process of phagolysis, as Metchnikoff maintains.

As regards the origin of the immune body the evidence is unanimous, showing that it originates from the lymphoid tissues, and probably from the mononuclear leucocytes. It is found that if animals be injected with cholera vibrios, killed at varying intervals afterwards, and extracts made of the different organs, the first sites in which the antibodies make their appearance are the lymphoid organs, especially the bone-marrow, spleen, and lymphatic glands. Similar facts have been observed for other bacteria and for red blood-corpuscles (Pfeiffer and Marx, Deutsch, Wassermann). And in Bulloch's experiments the amount of hæmolytic amboceptor was found to run roughly parallel with the number of mononuclear leucocytes present in the blood. In all probability this increase of circulating mononuclears must be regarded in such cases as an indication of the activity of the lymphoid organs, which are to be regarded as the main source of the protective antibodies. This is a generalization of the highest importance in immunity, and it is



fortunate that the evidence in its favour is clear and direct ; and it may be added that the proof is strengthened by the demonstration of the fact that the agglutinins are formed in the same region. It seems clear that the main, if not the only, function of the lymphocyte is the elaboration of the defensive antibodies.

There is no doubt that immune body, like the other antibodies, circulates as such in the plasma. The most striking proof is that of Metchnikoff in regard to spermatotoxin, but other evidence is available, and the point is not disputed.

### Methods of Researches on Immune Bodies and Complements.

The *methods* employed in the investigation of the hæmolytic sera are fairly simple in theory, though, as a rule, somewhat tedious in practice, owing to the necessity, in most cases, for quantitative work (so that slight degrees of hæmolysis may not be overlooked), and for numerous controls. The animal used in the preparation of the immune serum will naturally depend on the corpuscles to be employed, but in most cases the rabbit is most convenient. The corpuscles used for immunization should be collected in normal saline solution containing about 1 per cent. sodium citrate, centrifugalized, and rewashed three or four times in normal saline solution, so as to remove every trace of serum, complements, etc. If this is not done the resulting serum may be of great complexity, and results obtained by its action misleading. The emulsion used for the injection may contain about half its volume of corpuscles, and some 10 c.c. (or more in the case of large rabbits) may be injected into the peritoneum. In most cases three such injections at weekly intervals will cause the production of a powerful hæmolytic serum, but if there is any doubt 5 c.c. or so of blood may be withdrawn from the marginal vein of the ear, and used to test the progress of the immunization. The ear is well rubbed to make it hyperæmic, shaved, and washed with alcohol. A small puncture is now made with a flat surgical needle or fine knife, taking care that the vessel is merely incised and not completely divided. As a rule the blood will now flow quickly, drop by drop, and a sufficient amount may be collected in a sterile test-tube. If the flow is sluggish the blood may be milked out by passing the finger gently along the vein.

When the immunization is complete the rabbit is usually killed, and as much blood as possible is collected. The best way to do this

is to administer chloroform, and insert a cannula into the carotid artery; an easier method is to stick a small piece of capillary glass tubing through the wall of the heart whilst it is still beating, the animal being, of course, under chloroform. In either case the blood is led into a sterile tube, allowed to clot—preferably in the incubator, as the retraction of the clot is thereby increased—and the clear serum withdrawn by means of a sterile pipette, and stored in sterile tubes or small flasks, which may be plugged with cotton-wool or hermetically sealed. Where the serum is to be heated to destroy complement this may be accomplished in a thermostat, or more simply by sealing it in a narrow tube which is tied to the bulb of a thermometer, and placed in a beaker of warm water, and the temperature regulated by hand by the application and removal of a spirit-lamp. By this means any desired temperature can be maintained within very close limits, and the necessity for maintaining several thermostats or of altering the regulator is removed.

The emulsion of corpuscles to be employed in the actual testing is prepared as above, and must be rewashed at least four times, since it is found that traces of complement may remain after three washings. The emulsion is usually made up so that it contains 5 per cent. of corpuscles. This is readily accomplished by performing the last centrifugalization in a graduated tube, going on until the volume is constant. The bulk of the corpuscles is then read off, and, the necessary amount of normal saline solution being added, the corpuscles thoroughly mixed in. It is advisable that aseptic precautions should be observed throughout, but as this is troublesome it can often be omitted. It must be remembered, however, that many common bacteria produce hæmolysis, and that if the mixtures of corpuscles, serum, etc., be incubated for long periods fallacies may arise from this cause.

The actual experiment in its simplest form is carried out as follows: The necessary amounts of 5 per cent. emulsion of corpuscles, heated serum, and complement are placed in a narrow test-tube, and in most cases normal saline solution is added to bring the whole up to a definite volume. This is now incubated for two hours, being stirred or shaken once or twice in the meantime. It is now removed and placed in a vertical position in the ice-chest for twelve hours or so and examined. If there is complete hæmolysis the fluid will be deeply coloured, and there will be no sediment, or only a minute deposit of stromata. With partial hæmolysis the fluid will be less deeply coloured, and there will be

a deposit of undissolved corpuscles, and when there is no hæmolysis the fluid will be untinged.

Modifications of the process are numerous, and almost every investigator has his own. Thus it is very convenient to make the mixtures by means of one of Wright's pipettes. The whole is sucked into the pipette, which is sealed and incubated. At the end of the process the mixture may be expelled on to white filter-paper. Any unaltered corpuscles will form a solid dark deposit in the centre of the drop, whilst the fluid which soaks through the paper will be tinged or colourless, according to the amount of hæmolysis which has taken place. The amount of hæmolysis may also be determined more accurately by comparison of the supernatant fluid with a series of colour-standards previously prepared. The mere presence or absence of the phenomenon may be readily shown by dropping some of the fluid on to filter-paper.

The amount of immune body present may be determined by some such process as the following: In each of a series of narrow test-tubes (having a mark to indicate 2 c.c.) is placed 1 c.c. of the emulsion of corpuscles, and then varying amounts of the heated immune serum—*e.g.*, 0·001 c.c., 0·0025 c.c., 0·005 c.c., etc., or more if the serum be a weaker one. As these small amounts are not easy to measure accurately, the serum may be diluted ten or a hundred times with normal saline solution and suitable multiples, these amounts taken in the case of the smaller doses. The actual measurements are done with graduated pipettes, which can be procured from any instrument-maker. The complementing serum is then added: the amount necessary to dissolve 1 c.c. of fully-sensitized serum should have been previously determined by a few rough tests (we will suppose it to be 0·2 c.c.). Lastly, sufficient normal saline is added to bring the volume of each tube up to 2 c.c., and the whole series treated as above. Thus—

No.	Emulsion of Corpuscles.	Heated Immune Serum.	Fresh Serum.	Hæmolysis.
1.	1 c.c.	0·001 c.c.	0·2 c.c.	None.
2.	"	0·0025 c.c.	"	"
3.	"	0·005 c.c.	"	"
4.	"	0·0075 c.c.	"	Trace.
5.	"	0·01 c.c.	"	Partial.
6.	"	0·025 c.c.	"	Complete.
7.	"	0·015 c.c.	"	"
8.	"	0·0175 c.c.	"	"
9.	"	0·02 c.c.	"	"
10.	"	0·025 c.c.	"	"

Here 0.0125 c.c. of the immune serum contained sufficient immune body to sensitize fully 1 c.c. of a 5 per cent. emulsion of corpuscles—*i.e.*, a given volume of serum will sensitize 1.25 of its own volume of corpuscles.

The determination of the amount of complement is made by an inversion of this method. Thus Gay, who has made numerous investigations as to the amount of complement present in human serum, proceeds as follows: The sensitizing serum is derived from a rabbit which has been injected with ox corpuscles. This is heated, and the amount necessary for complete sensitization of a definite amount of ox corpuscles is determined; thus in his experiment 0.7 c.c. saturated 7 c.c. of a 5 per cent. emulsion. A series of tubes, each containing 1 c.c. of a 5 per cent. emulsion of fully-sensitized corpuscles, is prepared, and varying doses of the serum to be tested are added; the amount, which is small, is prepared by dilution with normal saline to such an extent that the actual bulk added is 0.1 c.c. The subsequent treatment is as above. Gay and Ayer find that on the average about  $\frac{1}{40}$  c.c. has to be added to bring about complete hæmolysis, the limits being  $\frac{1}{10}$  and  $\frac{1}{80}$  c.c.

Quantitative researches on the bacteriolytic action of the serum are very much more difficult. The actual determination of the amount of bactericidal action is by no means easy, and the results obtained are of very little importance, since the serum may be very deficient in complement, and deviation may occur. The method which has been chiefly employed is that of plating out after the bacteria and serum have been allowed to act together at incubator temperature for a given period. The method is briefly as follows: The emulsion of bacteria must be of constant strength. As a rule, it is sufficient to take a twenty-four-hour broth culture, and to dilute it to the same degree in all experiments; or the same loop may be employed throughout, or some one or other of the counting methods which have been described may be used. The emulsions should be dilute, so that all the bacteria may be killed. Klien recommends 1:8,000 of a twenty-four-hour broth culture in the case of *B. typhosus*. Lastly, normal saline solution is better than broth as a diluting agent, since it diminishes the chance of error owing to the multiplication of bacteria during the somewhat lengthy process of preparing the dilutions.

The actual process is as follows: Measured small amounts of the serum to be tested are placed in a series of tubes, a uniform

amount of the emulsion added, each tube made up to a definite volume, and all incubated for one to four hours. At the end of this period a uniform quantity is withdrawn from each, and plates prepared either by mixing with melted agar, or gelatin where suitable, or by smearing over ready-poured agar plates. The amount must, of course, be the same in each case, and may be easily withdrawn by means of one of Wright's pipettes, which is sterilized after use by being washed out several times with boiling water or oil at 150° C. The plates are then incubated, and the colonies which develop after twenty-four or forty-eight hours are enumerated, and the amount of serum which kills all or the greatest number of bacteria is noted.

Certain controls are necessary, the main being—(a) a tube inoculated as above, but without the addition of serum; and (b) a tube also containing bacterial emulsion, and also a relatively large amount of heated serum. The main error comes in from the reduction of the number of colonies in consequence of agglutination, but this can be discounted in some measure by comparison with the plate prepared from control (b).

Other methods are employed, notably that of Wright, for which the original article should be consulted. The value of the processes is not great, since it does not tell us even the actual bactericidal value of the circulating blood (since we do not know the amount of complement which is available) nor the amount of immune body. In some cases a serum containing a large amount of the latter substance will show little or no bactericidal power *in vitro*, owing to the deficiency in complement, and may require the addition of a hundred times its volume of normal serum to be fully complemented.

To determine the relative amount of immune body present, the principle of the method used for the measurement of the hæmolytic amboceptor is adopted, a series of mixtures of constant amounts of bacterial emulsion and fresh normal serum is prepared, and varying amounts of the heated immune serum to be tested are added, the whole made up to uniform volume, and treated as above. Here a further control is necessary, since the fresh normal serum may contain some immune body or be otherwise bactericidal. One of Neisser and Wechsberg's examples of this process has been already quoted.

The determination of the amount of bactericidal complement is simple enough theoretically, and follows the same lines as that

for the determination of the hæmolytic complement. In actual practice these procedures are all so tedious that most of the measurements of complement have been made on the latter variety; the two are believed to have the same origin, and there is no reason to think that the one does not run parallel to the other. Gay and Ayer employ a more direct method, adding varying amounts of the serum to be tested to a definite volume (0.5 c.c.) of a suspension of cholera vibrios, prepared by emulsifying four twenty-four-hour agar cultures in 10 c.c. of normal saline, and subsequently adding a sufficient sensitizing dose of serum from an immunized rabbit. The action is allowed to go on for one and a half hours at 37° C., films prepared, stained, and examined as to the degree of the changes undergone by the vibrios. They found that  $\frac{1}{200}$  c.c. of normal human serum was sufficient to cause a complete Pfeiffer's reaction in 0.5 c.c. of cholera emulsion tested as above, whilst when  $\frac{1}{1000}$  c.c. was used there were distinct changes.

### The Cytolysins.

Bordet's discovery of acquired hæmolytic powers, arising from the injection of foreign red corpuscles, proved the starting-point of a most interesting series of researches, for it was soon shown that the phenomenon was not an isolated one, but that it might be produced when almost any animal cell took the place of the red corpuscles. Thus, Metchnikoff in 1899 prepared a *leucotoxic serum* by the injection of the cells from the spleen of a rat (mostly lymphocytes) into a guinea-pig. The serum of the latter agglutinated and partially dissolved the leucocytes, the lymphocytes being most affected. Besredka studied the subject, and found that, as in the hæmolysins, two substances—one thermostable (sensibilatrice or amboceptor) and one thermolabile (alexin or complement)—took part in the reaction. He studied the specificity of the substance, and found it was not sharply specialized in its action to leucocytes of the animal used for the source of the antigen; it would attack those of most animals, but not man. It was toxic, 3 c.c. of serum being a lethal dose. He also prepared an antileucotoxin.

The next cytolyisin to be prepared (by Landsteiner, and independently by Metchnikoff) was *spermotoxin*. This was a very suitable subject for study, since its action could be readily

observed, the cells on which it acted being motile ; and it must be pointed out that these cytolytins do not cause complete solution of the cells. A red blood-corpuscle is a remarkable object, and macroscopic evidence of its (partial) solution is easily obtained. It is otherwise with the cytolytins, and here refined histological methods are often necessary for the demonstration of a solvent action. Agglutination of a suitable suspension of the cells is, however, invariably present, and is easily observed. Further evidence is also obtainable by observing the action of the serum on live animals and the disturbances in function which it produces. In the case of the spermotoxin, the spermatozoa are rendered immotile, and are agglutinated, but are not dissolved.

Several interesting phenomena were brought to light by a study of spermotoxin. Thus, Moxter showed that its action is not sharply specific, since a spermotoxic serum is also hæmolytic. Metchnikoff thought that this non-specificity is only apparent, since hæmolytic sera are not spermotoxic ; and he succeeded in removing the hæmolytic substance from the serum by the addition of red corpuscles, leaving the spermotoxin intact.

It may be pointed out here that similar results have been obtained with the other cytolytic sera ; they are not sharply specific, all being hæmolytic, and some attacking several cells, as well as those which have been used as their antigens. This subject has been thoroughly investigated by Pearce. Some of his results may be briefly epitomized. Hæmolytic sera act, of course, most strongly on the red corpuscles, which they lacerate, and give rise to hæmoglobinuria. They also produce fatty degeneration of the renal epithelium and necrosis of the cells of the liver. With very small doses there may be no hæmoglobinuria, bile-pigment being present in the urine, but the lesions of the liver and kidney are also present. A serum prepared by the injection of kidney cells, thoroughly washed, so that no blood was injected with them, was hæmolytic *in vitro*, but did not produce hæmoglobinuria. It caused albuminuria, with presence of casts and granular degeneration of the liver cells. A serum similarly prepared from the suprarenal glands had no action on them, but produced granular or fatty degeneration of the kidney and liver. An animal injected therewith showed immediate pallor of the mucous membranes and cardiac and respiratory failure. He found that hepatotoxins and pancreatotoxins were without specific action, behaving simply like hæmolytins.

It is obvious that these results are readily explicable if we assume that the red corpuscles and tissue cells have receptors in common, but that a particular sort of receptor is most abundant in a particular species of cell. But, according to Beebe, sera which are much more sharply specific can be prepared if, instead of injecting the cells themselves, we employ the nucleo-proteid prepared from them; the method had also been employed by Bierry and Pettit in the case of the nucleo-proteids of the liver and kidney.

Another serum which was prepared early in the history of the subject was *trichotoxin*, the cytotoxin for the ciliated epithelium. This also, as Von Dungern showed, had a hæmolytic action, though he considered that there were no red corpuscles in the substance used for the injections.

*Hepatotoxin* is produced by the injection of emulsions of liver cells or of nucleo-proteid prepared from the liver. It causes congestion of the liver, fatty or granular degeneration of the protoplasm, and dilatation of the bile canaliculi. If the serum has been prepared by means of nucleo-proteid, no other organ is affected. But the effects of hepatotoxin may also be produced by nephrotoxic and lienotoxic serum, etc.

A considerable amount of interesting work has been done on *nephrotoxin*, and the questions which have arisen are far from having been settled. It is produced in the usual way, by injection of animals with a fine emulsion of kidney cells (well washed to remove blood-corpuscles, etc.) from a foreign species. It produces albuminuria (but no glycosuria, according to Bierry), and symptoms having at least some resemblance to uræmia (coma, etc.) are occasionally produced. These symptoms are not specific, and are frequently caused by injections of other cytolytins (nephrotoxin, etc.), or of emulsions of foreign cells. We have already pointed out that Beebe and others have claimed to be able to produce a truly specific nephrotoxin by means of injections of nucleo-proteid from the kidney.

Of more interest is the question of the possible formation of an autonephrotoxic body, which might conceivably be produced when part of a kidney becomes disorganized whilst in the living body. It has been thought, for instance, that when a toxin acts on the kidneys it produces death and subsequent solution of the renal epithelium, and that these soluble substances, being absorbed into the system, call forth an autonephrotoxin, which reacts on



the kidney, dissolving more cells, which produce more of the antibody, a vicious circle being thus produced. Hence a pathology for nephritis and uræmia on quite new lines was suggested by Ascoli and Figari and Lindeman, etc. Thus the cardiac hypertrophy of renal disease is supposed to be due to a spasm of the peripheral vessels and increase of blood-pressure due to the nephrotoxic serum; the nervous symptoms on the supposition that there is a neurotoxin produced concurrently with the nephrotoxin, and spontaneous recovery by the production of an anti-auto-nephrotoxin, a substance for the existence of which there is a little evidence.

There is a certain amount of experimental confirmation of this theory. Thus Lindeman treated dogs with potassium bichromate, causing nephritis, and found that the serum of these animals (though free from bichromate) was toxic for other dogs. Again, Le Play and Corpechot found that the injection of renal tissue (of the guinea-pig) into the rabbit produced important organic lesions: great increase in volume, fibrosis of the connective tissues, cystic dilatations of the tubules, and desquamation of the renal epithelium. That these changes may be due to the production of a nephrolysin appears possible from the fact that when these injections are made in gravid animals similar appearances may be seen in the kidneys of the fœtus, suggesting that the nephrolysins traverse the placenta (Charrin and Delaware). Albarran and Bernard also found that renal tissue is lethal on injection, but Pearce denies this, and holds that their animals were killed by bacterial infection. Further, Nefedieff ligatured one ureter (in the rabbit), and found changes similar to those seen in chronic nephritis. His results might, of course, have been due to the formation of a nephrotoxin in consequence of the disintegration of the renal cells subsequent to ligature of the ureter; but Albarran pointed out that, according to Nefedieff himself, the second kidney was unaffected at a time when the serum was nephrotoxic, as tested on other animals. Sheldon Amos failed to reproduce Nefedieff's results; according to her, ligature of one ureter causes death after an average period of sixty-nine and a half days in the guinea-pig, and fifty-two days in the rabbit. There may be lesions on the control side, but if so these are slight, and the liver is also affected. But that these results are due to the action of a nephrotoxic serum appears most unlikely, from the fact that when the whole pedicle of the kidney, or the

artery and vein, are ligatured, no such results follow, though the whole substance of the kidney is absorbed. These and other researches make it very doubtful whether the facts observed in nephritis are explicable on the nephrotoxic theory alone, but further information on the subject is needed.

The degree of specificity of the nephrotoxic serum is not yet settled. According to Pearce, the lesions which it produces may be caused by other sera. This has been confirmed by other observers, but Woltmann, though in accordance with Pearce on the main question, thinks that nephrotoxin does exhibit some degree of specificity: it produces marked congestion of the medulla and swelling of the cortex, results not seen with other sera. Beebe also finds nephrotoxic sera produced by the injection of nucleo-proteid prepared from the kidney cause renal lesions, whereas other cytotoxic sera produced by the injection of other nucleo-proteids do not.

*Gastrotoxic* serum is especially interesting in view of its possible action in the production of gastric ulcer. It has been very thoroughly studied by Bolton, and was prepared by injecting rabbits with emulsions or extracts of guinea-pig's gastric mucous membrane into the rabbit. The serum thus obtained was injected into guinea-pigs, and was found to be lethal, even in small doses (1 to 5 c.c.); a dose of 10 c.c. usually caused death in twenty-four hours. The lesions were confined to the stomach, and were striking and characteristic. They consisted of patches of necrosis extending down to the muscularis mucosæ, and often surrounded by a hæmorrhagic infiltration of the surrounding tissues. After a time this necrotic tissue disappeared, leaving an ulcer presenting some resemblance to the ordinary acute gastric ulcer. These appearances (necrosis, etc.) were not seen if the acidity of the gastric juice was neutralized by alkalis. No very definite action could be demonstrated on gastric mucous membrane *in vitro*, but isolated cells exposed to the action of the serum became hyaline in appearance, resembling shadows. Further, the serum had a powerfully agglutinating action on gastric cells, and produced a precipitate in clear solutions obtained by filtration through a Berkefeld filter.

Interesting facts were discovered as regards its specificity. It is hæmolytic, but this appears to be due to the fact that it contains hæmolysin as well as gastrotoxin. This is shown as follows: If the serum is heated it loses its power to produce the

characteristic necrosis of the stomach, so that its immune body cannot be reactivated by guinea-pig alexin; but the latter body can reactivate hæmolyisin prepared by immunizing rabbits with guinea-pig's corpuscles. If the serum is placed in contact with an emulsion of guinea-pig's mucous membrane, it becomes innocuous, both immune bodies being absorbed; but when saturated with red corpuscles, it loses its hæmolytic power, and retains its necrotizing properties.

Rabbits injected with emulsions of rabbit's mucous membrane develop a gastrotoxin which acts on guinea-pigs, but not on the rabbit itself. Similarly for guinea-pigs treated with emulsions of mucous membrane from the same species: their serum becomes gastrotoxic for the rabbit, not for the guinea-pig.

To account for these remarkable facts it is suggested that the gastrotoxin has two cytophile groups—one which combines with the gastric cells of the animal which produces it, and one which combines with those of the other species. Thus the gastrotoxin of the rabbit has a cytophile group, *a*, which has an affinity for rabbit's gastric cells, and a second, *b*, which unites with those of the guinea-pig. During the process of immunization the animal produces an anti-immune body, which combines with the cytophile group *a*, but not with *b*. This is readily explicable on the side-chain theory. It follows, therefore, that the gastrotoxin is never efficacious against the species which produces it, being always neutralized as regards these cells by a partial anti-antibody.

*Anti-intestinal serum* has been prepared. It is extremely toxic, causing gangrene of the mucous membrane and death. Less powerful sera cause non-fatal diarrhoea.

*Syncytiolysin*, or *placentolysin*, has been obtained by injections of emulsions of placental tissue. According to Liepman the serum thus obtained will give a precipitate with a solution of placental tissue, with blood from the umbilical vein, or even with that of a gravid woman, but not that of a non-gravid woman or a man; hence he proposed a serum test for pregnancy. But his results, which seemed highly improbable, have been disproved by Weichardt, who showed that the serum thus obtained acts equally well on placental solutions and on all human blood. The question of the action of the placenta when injected (in a fine emulsion) into the tissues is of some importance in connection with a possible pathology for eclampsia and the nephritis of pregnancy. Most authorities (though not all) find that the animal thus treated

develops nephritis and lesions of the liver. Now it is known that in some cases at least fragments of the placenta break loose and circulate for a time in the blood during pregnancy, and it is not difficult to suppose that dissolved products of these cells are constantly being absorbed. Hence it seems possible that some at least of the cases of nephritis during pregnancy and of eclampsia may be produced in this way; and Weichardt produced symptoms resembling those of eclampsia by macerating placental tissue with syncytiolysin, and injecting the result into normal rabbits. Hence it was hoped that an antitoxin for puerperal eclampsia and nephritis might be produced by immunizing animals with placental tissue, so as to produce a serum which would dissolve the circulating placental cells, and prevent the destruction of the cells of the liver and kidneys. This does not seem to have been put into practice, and there are numerous theoretical objections which might be raised.

*Prostatotoxin* has been prepared by Jungano by injecting an emulsion of the prostates of young dogs into rabbits. The serum clumps emulsions of prostatic cells, and when injected *in vivo* produces fatty and granular degeneration of the epithelial cells of the gland and a leucocytic infiltration of the stroma; it is apparently fairly specific, there being no obvious lesion of other organs.

*Neurotoxin* has been prepared by Delezenne, Centanni, Delille, and others, by the treatment of one animal with the brain substance from another, which is often in itself somewhat toxic, so that the process does not always succeed. It causes a remarkable series of phenomena indicative of profound intoxication of the nerve centres. These usually begin with somnolence and torpor, which come on shortly after the injection, and may last some hours, being succeeded by convulsive crises, in which there are tonic and clonic spasms; there may be one such attack, or a series, with coma between each. The temperature is lowered, and death usually occurs in one to twenty-four hours. The histological changes are marked, and affect the ganglion and cortical cells; they indicate a profound degree of destruction of these structures (neurolysis). The substance is most active when injected into the brain direct; when introduced into the veins it is innocuous, but forms an anticytolysin.

Schmidt has prepared a serum which he claims to be more or less specific for the *peripheral nerves*. A guinea-pig which is injected

with an emulsion of the sciatic nerves of frogs develops in its serum a substance which leads, when injected into frogs, to the rapid production of symptoms of paralysis, which may become complete, and resemble Landry's paralysis in man. Most of the animals die in from twelve to forty-eight hours, and their nerves show fragmentation of the axis cylinders, multiplication of the nuclei in the sheath of Schwann, etc. The serum is also hæmolytic for frog's corpuscles, but neither normal serum nor a simple hæmolytic serum produce these paralytic symptoms.

The suggestion has been made that sympathetic ophthalmia might be due to a specific cytotoxin formed by the disintegration and absorption of the iris and ciliary body in the injured eye (Bram Pusey). There is a certain amount of experimental proof in favour of this interesting theory. Thus Le Play and Corpechot prepared an ophthalmotoxic serum, and found that animals injected therewith were less resistant than normal animals to injections of *B. pyocyaneus* into the anterior chamber. The subject has been more fully investigated by Golovine, who prepared his serum by injecting into rabbits an emulsion of the ciliary bodies of the dog (twelve to twenty in each animal). The ophthalmotoxic serum thus obtained was tested by injection into the anterior chamber. It led to the production of a slight pericorneal injection, a fibrinous exudate into the anterior chamber, and some appearances of iritis. Microscopically it was found that the ciliary processes presented evidence of inflammation and degeneration, being infiltrated with leucocytes containing granules of pigment. There was also marked evidence of degeneration of the epithelium covering these processes. When the serum was injected into the veins the macroscopic effects were not observed, but similar microscopic changes were noted in the epithelium.

The pigment taken up by the leucocytes was derived from the ciliary processes, which may become almost absolutely decolourized. Hence Golovine holds that his serum contains not only a specific cyclotoxin, but also a pigmentolysin.

Other cytolytic sera have been prepared, but are not of much interest. A reference may be made to thyrotoxic serum, which has been used in the treatment of exophthalmic goitre, though without any considerable success. Indeed, the use of cytolytic sera has proved most disappointing in practice. An anti-epithelial serum which was very early suggested as a cure for cancer, but proved inefficacious, and others have been tried. There are very

many problems connected with cytolytic action that are unsolved, and there can be but little doubt that future research in this direction will yield results of great pathological importance, both in theory and in practice, and whether the therapeutical advance will take the form of a potent serum or of a juster knowledge of the inner processes of the body in disease the future will show.

#### THERAPEUTIC APPLICATIONS OF BACTERICIDAL SERA.

The discovery of the great therapeutic value of diphtheria antitoxin naturally led to attempts at antitoxin treatment of other diseases, but it was soon found that it was impossible to prepare a potent toxin, and therefore antitoxin, in the great majority of cases. The discovery of Pfeiffer's phenomenon, and the subsequent researches on bacteriolysis and hæmolysis, with the demonstration of the nature of substances at work, indicated that the problem was to be solved, if at all, on other lines, and antisera were made by injecting the bacteria themselves into suitable animals. The process need not be described at length, and of course slight modifications are necessary in different cases. In general the early part of the treatment consists in the injection of small doses of dead or avirulent bacteria, or in some cases (*e.g.*, anthrax) of a more virulent vaccine and of a protective serum from an already immunized animal. The animal (horses, donkeys, or goats, are usually employed) is thus immunized, and now large doses of virulent bacteria are given in order to stimulate the production of antibodies to as great an extent as possible. This part of the treatment is often prolonged, and may last for a year or more. At the end of this time the animal is bled in the manner described above, and the serum used for protective or curative purposes. In some cases it is standardized, the usual method being to determine the amount which will just protect a small animal from a lethal dose of living bacteria, or from some multiple thereof. Thus Sclavo's serum is tested by injecting 1·6 c.c. into six rabbits, each of which receives shortly afterwards a known dose of virulent bacilli; if three of the animals survive, and the rest have their lives greatly prolonged (as compared with controls), the serum is considered to be efficacious. Antistreptococcic serum may be standardized in a similar way: according to Hewlett, 0·05 c.c. should suffice to preserve a rabbit from ten lethal doses of living streptococci injected intravenously. In other cases a somewhat more refined method is adopted, and the

amount of antibody present is estimated. In the case of anti-typhoid serum the simplest method is to measure the degree of agglutination, which may rise as high as 1 : 1,000,000. This cannot be taken as an *absolute* criterion of the amount of bactericidal substance present, but in the great majority of cases the two antibodies are developed roughly proportionately, and the agglutination may be taken as a fair guide. Of course, the bacteriolytic potency may be worked out by the method already described, taking care that a sufficient amount of complement is added, and that there is no deviation. This is probably the best method, and is sometimes employed; thus Shiga found that 0.0001 c.c. of his antidyentery serum when reactivated by 0.3 c.c. of fresh serum would kill all the bacilli in  $\frac{1}{300}$  milligramme of a one-day-old agar culture.

The results of tests of this nature have been to show that extremely potent sera can be obtained against typhoid bacilli, cholera vibrios, dysentery bacilli, and perhaps streptococci; sera of less but still of some power against plague bacilli, anthrax bacilli, pneumococci, the gonococcus, and the meningococcus; whilst the results with staphylococci and tubercle bacilli have been to all intents and purposes negative.

The method of action of these sera is not quite settled. In some cases there is an abundance of bactericidal immune body, and there is no reason to doubt that, when employed as a prophylactic agent, this becomes complemented in the animal body, and causes bacteriolysis of the infecting organism. This is certainly the case with the sera directed against typhoid fever, cholera, and dysentery. In other sera, which are, nevertheless, of definite protective and even curative value, this effect cannot be demonstrated. This is the case with anti-anthrax serum. Here we have to assume either that the substance owes its value to the presence of opsonins or of anti-endotoxin, or possibly (in some cases) that it may contain free toxins, or at least specific antigens, and act as a vaccine, producing active rather than passive immunity, as was suggested by Wright in the case of Calmette's typhoid serum, which is prepared in a manner somewhat different from that just described. There is some reason for thinking that Sclavo's serum acts opsonically, and with regard to the presence of anti-endotoxin, it may be pointed out that the *prolonged* course of immunization usually employed may lead to the production of this substance in small amounts.

These sera, which for the purpose of convenience we shall consider together as if they were all bactericidal, are in general protective, but not curative. Thus the clinical use of antityphoid and anticholera serum has shown them to be quite worthless or even dangerous; dysentery serum is of distinct value if used early in the attack, and some of the other sera are of some value, and their use is discussed in the final section of this book. In general terms, however, and comparing them with diphtheria antitoxin, we may say that they have proved most disappointing in practice. The reason for this failure requires some discussion.

Antibacterial sera as ordinarily used are, of course, devoid of complement, which has usually disappeared long before use; it is rendered inert on keeping, and is especially susceptible to the antiseptics commonly added as a preservative. The first suggestion is that the failure of the serum is due to lack of complement: the union of the amboceptor and bacterium is supposed to take place as usual, but the necessary alexin is not forthcoming. This may be due to one of two causes: in the first place, there may be (as is known to occur in certain diseases) a deficiency in the amount of complement in the serum; in the second place, that which is there may be unsuitable in nature.

As regards deficiency in complement, this has been found to occur in certain diseases, and is very probably a common occurrence in pathological conditions and states of malnutrition in general; but when we consider the comparatively small amount necessary to activate a large dose of sensitized bacilli, there is no reason to think that it ever falls below that level. Again, the facts known concerning the immunity of the dog and other animals to anthrax are of such a nature as to render it improbable (in this case, at least) that deficiency of complement can really be of much importance; for dog's serum contains abundance of amboceptor, yet no suitable complement, and is devoid of bactericidal action. We shall see reasons for believing that amboceptor may possibly act as opsonin, in some cases at least, without the concurrence of complement, and this is probably the explanation of the immunity of the dog to anthrax.

The other explanation, that of Ehrlich, is that the complements present in human serum may be unsuitable to reactivate serum derived from a horse, ass, or goat, or other animal used as the source of the immune body. To obviate this, he has proposed the use of sera from several species of animals, in the hope of



finding one that can be reactivated by human complement, and has suggested the use of serum from the higher apes, the complements of which closely resemble those of man. These explanations are not very satisfactory. Thus Shiga's antidysentery serum is certainly readily complemented by human blood; and although it has certainly some beneficial action, it is useless in the chronic stages of the disease, and this although the amount injected must be very much greater than is necessary to dissolve all the bacilli present in the body.

Another possible cause of failure is the deviation of complement. If we admit the action of the bactericidal substances—by no means undisputed—in the natural process of recovery from disease, we can easily see how it is that this process does not occur under normal conditions. Thus, when infection with the typhoid bacillus occurs, there is at first little or no amboceptor in the blood. The small amount present is quickly seized by the bacteria and removed, and although a few bacilli may be killed, the great majority flourish unchecked. But amboceptor is soon put out in gradually increasing amounts, and at first is used up as soon as it is formed. The two processes, proliferation of bacilli and increase in the amount of amboceptor, now progress side by side, and on their relative rapidity depends the outcome of the disease. At first bacillary proliferation takes place more rapidly than the production of antibody, and the symptoms gradually become more and more severe. After a time the antibodies are released in larger and larger amount, and (in a favourable case) a time arrives when there is exactly enough for all the bacteria present. We must assume that enough complement is available, and in this case it is easy to see how it can never become deviated; for all the amboceptor is rapidly linked up to the bacilli, and does not accumulate in excess in the blood. It does seem possible, however, that an accumulation of amboceptor might conceivably determine a relapse, bacteria which escaped destruction owing to their having lain in the tissues, gall-bladder, or other inaccessible region, being now free to grow in the blood owing to the removal of complement by deviation.

But when a dose of bactericidal serum, containing, it may be, many times more immune body than is necessary for the solution of the bacilli present, is suddenly thrown into the circulation, the conditions are quite different. Here there is an excess of

immune body relatively both to the bacteria and to the complement, and deviation of the latter may occur. Hence it is at least conceivable that a dose of bactericidal serum may be injurious in that it actually inhibits the normal bacteriolytic processes that are at work in the blood-stream. We have already quoted processes exactly parallel in describing the experimental proof of the deviation of complement.

Another suggestion that has been made is to use perfectly fresh immune serum, or to reactivate it by fresh serum from a normal animal. But this seems not to be successful, and apparently alien complements rapidly unite with the tissues of the animals into which they are injected, and so become inert.

It would seem that no explanation based on deficiency in complement will be found satisfactory: the facts concerning the action of the dog's serum on anthrax bacilli appear to offer a crucial experiment settling this point. Nor if the added amboceptor really acts as opsonin would the question of complement come in. The most satisfactory explanation appears to be that the sera do not actually come in contact with the bacteria in the lesions, though they may, and very probably do, tend to sterilize the blood, and so prevent further generalization of the infection. This question—of the accessibility of the bacteria in the lesions to the substances circulating in the blood—is probably one of prime importance in immunity and recovery, and we shall meet with it again in dealing with the opsonins. It seems to meet the facts of the case very well with regard to the action of the serum in dysentery. In acute cases it is of value; and here the bacilli are lying in regions which are fairly accessible to the blood. In chronic dysentery it is almost useless, and in this form of the disease the bacilli are shielded by a dense and impermeable layer of inflammatory tissues. And in cholera the bacilli are mostly lying in the intestinal tract; probably a few do gain access to the blood and tissues, and are immediately destroyed. In typhoid fever the bacilli are found in the blood early in the disease, and later, roughly at the period at which antibodies make their appearance in large amounts, they disappear. But there are always large numbers in the lymph glands and spleen, regions in which it is almost certain they are shielded from the action of the blood. This explanation appears far more satisfactory than any depending on deficiency in complement.

If the bacteria in the blood-stream are actually dissolved by the

added bactericidal substances, a new danger is involved—that of the liberation of a large amount of endotoxin. This substance we believe not to be liberated when bacteria are dissolved within the leucocytes, but to be set free when extracellular solution takes place. The essential fever of the early stages of typhoid fever is very probably due to the endotoxin set free by the solution of the bacilli in the circulating blood, and any sudden addition to this amount occurring before the tissues have become immunized or trained to produce anti-endotoxin may be fraught with danger. There can be little doubt that if sero-therapy has any future triumphs in store, they will be in the direction of the production of anti-endotoxins.

The various antibacterial sera in common use are considered in the last section.

## CHAPTER VIII

### THE AGGLUTININS

AN exceedingly interesting and important group of antibodies, which were discovered by Gruber and Durham in 1896 (though their effect had been observed by Charrin and Roger in 1889 in the case of *B. pyocyaneus*),<sup>1</sup> are called the agglutinins, since they have the power of agglutinating their antigens, or causing them to adhere in masses. Their effect is best seen after the addition of the serum of a patient convalescent from typhoid fever (or of an animal which has been injected with typhoid bacilli) to a living culture of the organism. The bacilli, which at first are actively motile and are distributed uniformly throughout the fluid, first lose their motility, and then individuals may be seen to move nearer and nearer to one another, until they come into close contact. It often happens, especially in weakly agglutinating sera, that this approach of two bacilli may be seen to occur before their paralysis has taken place. They then revolve rapidly round a common axis, giving the observer the impression that they are united together by a sort of invisible link, which they struggle to break. This process continues, and fresh individuals are attracted to the groups, until at last all the bacilli, instead of being scattered equally throughout the fluid, are collected into masses, the intervening fluid being free. The process may also be watched with the naked eye, and the emulsion, which is at first uniformly turbid, will be seen to lose its homogeneity, and take on a finely granular appearance. This at first can only be realized by comparison with a control specimen to which no serum has been added, but in a little time it will be obvious that flocculi of bacilli are being formed, and that between these flocculi the fluid is clearing. Soon

<sup>1</sup> The effect had also been observed by Metchnikoff in the case of *V. Metchnikovi* in 1891; he was inclined to regard it as a general phenomenon, but failed to find it in another case. Similar appearances had also been seen by Issaëff in 1893.

(if the emulsion is thick enough) all the organisms will be found to have collected into a single mass or a few masses, the rest of the fluid being quite clear. Finally, these masses will sink to the bottom of the vessel, and it will be noted that if the bacilli in the control specimen also sink (as happens with killed organisms), the masses will be much more voluminous than the deposit of unagglutinated bacteria. A microscopic examination of the deposit in the two cases will show why this is. In the deposit of dead bacilli the separate rods have sunk down slowly, and have packed themselves closely together, and will be found, to a very large extent, to lie horizontally side by side. In the agglutinated mass the bacilli point indifferently in all directions, and the explanation suggests itself that they have been drawn forcibly together by a centripetal force, and have not had time to adapt themselves so as to take up as little room as possible. A result of this is that it is easy to distinguish between a specimen that has agglutinated and one in which the bacilli have simply settled, even although the actual occurrence of the phenomenon has not been witnessed.

The reaction is given with the serum of immunized animals, and is a general one. It is given with nearly all species of bacteria, though to a very different extent in different cases, with red blood-corpuscles, leucocytes, and with cells of all kinds. The occurrence of motility is not necessary for it, and dead bacilli will clump almost or quite as well as living ones. The reaction is, in general, specific, and a serum which is strongly agglutinating as regards one species of organism may be entirely devoid of action on others. Hence it was proposed by Gruber and Durham as a test for the identification of bacteria, and is of great value. Thus, when a bacteriologist has isolated a culture of an organism resembling *B. typhosus* from a patient suspected of having typhoid fever, or from a sample of water supposed to be contaminated, the first step in the identification is made by observing whether it is clumped by a serum known to have agglutinating powers over typhoid bacilli and not over others. Other tests are necessary for its complete identification, but these are slower, and for some purposes unnecessary. The clinical diagnosis of cholera by means of cultures from the stools is carried out in the same way, and sera adapted for either purpose can be obtained commercially.

The reaction, however, is not an absolutely specific one, and it is found that a given immune serum may clump not only the culture used in its production, but also those of closely allied

species. Thus typhoid serum clumps *B. coli*, the paratyphoid and paracolonic bacilli, the *B. psittacosis*, and others. This is called a *group reaction*, and is of profound interest in classification. It is not, as might be thought, a hindrance to the practical application of the process as a method of identification of the nature of a culture, since it is found that the action is exerted much more strongly on the organism used for the immunization than on others. This is determined by ascertaining the dilution necessary to bring about agglutination in a certain time at a given temperature. For example, we may find that certain specimens of anti-typhoid serum will agglutinate typhoid bacilli at a dilution of 1 : 10,000 in one hour, whilst *B. coli* is not affected if the dilution is greater than 1 : 50. In the practical use of this serum we should not be certain that a given culture was one of *B. typhosus* unless it reacted at 1 : 1,000 or more.

The explanation of these group reactions on Ehrlich's theory offers no difficulties. Agglutinin is, as will be shown, a specific antibody to the molecules of protoplasm contained in the bodies of the injected cells. In each cell these will be of many varieties, and to each a specific antibody will be produced. We must imagine a typhoid bacillus as containing a large number of one particular sort of molecule, a smaller one of another, whilst in the colon bacillus these relations will be the reverse. A typhoid serum, therefore, will contain much agglutinin which acts on the typhoid molecules, and a little which acts on a few of those present in *B. coli*; it will agglutinate the former strongly, the latter feebly. But the colon serum will contain antibodies to a few only of the molecules present in the typhoid bacilli, and will clump it only in strong dilutions.<sup>1</sup>

Agglutinins are formed, as we have seen, as the result of the

<sup>1</sup> There are a few noteworthy exceptions which have been recorded to these general rules. In a few cases of tuberculosis the power of agglutinating *B. typhosus* has been seen to rise, and Park has quoted a case in which an animal immunized against staphylococci increased its power against the same bacillus from 1 : 10 to 1 : 160. In interpreting these results we must always wonder whether they might not be explained by a rise in the sensitiveness of the culture used. But this objection does not apply to the observations of Posselt and Sagasser, who obtained an agglutinin which acted on bacteria other than those used for the injection, and which was not removed from the serum by these bacteria. And some cases have been recorded in which a serum had less action on its own antigen than on others. All these exceptions are rare and not fully investigated, and do not affect the general law.

injections of their specific antigens. They are also frequently present apart from any interference. For example, normal human serum clumps the second vaccine of anthrax powerfully, and in most cases has a feeble action on both *B. typhosus* and *B. coli*. Horse serum is very rich in agglutinins, clumping typhoid and coli bacilli, the *B. pyocyaneus*, and the cholera vibrio, often in dilutions as high as 1 : 100. In most cases agglutinins are present in small amounts in the serum of infants and young children, and become more abundant in later life. This suggests that they may be formed—in part, at least—by a process of auto-inoculation with bacteria, principally, perhaps, from the intestine. We have already seen, however, that on Ehrlich's theory the presence of antibodies in normal animals is readily explicable without such assumption.

The injections of bacteria or cells of any sort leads to the production both of agglutinins and of cytolytins, and in most cases of hæmolysis or bacteriolysis agglutination occurs as the first step in the process. The question arises, therefore, whether they are the same substance. It is easy to show that they are not, since sera which contains agglutinin do not necessarily contain immune body, or *vice versa*. In sera obtained by artificial immunization, of course, the two are almost invariably formed side by side, and it is only by special processes that we can obtain the one without the other. Thus Frouin claims that if dried dog's corpuscles are washed with acetone and injected into a rabbit, they cause the production of agglutinin; but no hæmolysin. The residue from the evaporation of the acetone, on the other hand, yields hæmolysin, but no agglutinin. But in sera from normal animals it is quite common to find the one without the other. Thus the serum of healthy human beings frequently clumps normal human corpuscles, but hæmolysis is extremely rare. The converse process—hæmolysis without agglutination—also occurs; and with regard to antibacterial sera of artificial origin, Fränkel and Otto found that when a dog was fed on typhoid cultures it developed agglutinin, but no immune body. Lastly, in many cases the action of agglutinin is destroyed at a lower temperature than that of immune body, although both substances are in a marked degree thermostable. We shall have to discuss the effects of heat on agglutinin more fully subsequently.

There is, as a matter of fact, a kind of antagonism between agglutination and cytolysis. Cells which are crowded firmly together are naturally shielded, more or less, from the solvent

action of the fluid in which they are suspended; and equally naturally cells which are dissolved do not show ordinary agglutination, though, as we shall see, they show a similar phenomenon.

The formation of agglutinins follows laws similar to those governing the formation of other antibodies. After each injection there is a negative phase, followed by a rise, which, as a rule, attains its maximum in about a week. In the case of typhoid fever no agglutinin can be demonstrated, as a rule, during the first week; there is then a steady rise, which usually attains its maximum at the commencement of convalescence. After this the amount tends gradually downward, and disappears after a time, which varies between a few months and several years. On a single occasion the author has seen a marked drop in the amount precede a relapse, during which a second rise occurred. This was obviously a negative phase, and the occurrence of the relapse might have been foretold therefrom.

Bacteria which have been acted on by agglutinin are not altered thereby in appearance, viability, or virulence, and the process does not appear to play a part of much importance in immunity. Two suggestions have been made in this respect: Gruber thought it caused the outer layer of the bacillus to swell up, so that it could be attacked by alexin, and Walker suggested that the clumping of the bacilli might render them more easily taken up in large numbers by the leucocytes. Possibly, also, the *paralysis* is the essential feature of the process, as a reaction of immunity, since we should expect non-motile bacteria to be more easily ingested by phagocytes. It is interesting in this connection to notice that the bacteria for which strong agglutinating sera are obtainable are all highly motile (*B. typhosus*, *coli*, and *pyocyaneus*, vibrios). The recent researches on the thermostable opsonins have caused a certain amount of attention to be directed to the agglutinins from this point of view, but nothing is definitely proved.

That agglutinin, in common with the other antibodies, unites directly with its antigen may be shown in several ways. In one an agglutinating serum cooled to 0° is added to a culture similarly cooled, and the mixture kept on ice. The bacteria will gradually settle down without agglutinating, and the supernatant fluid may be pipetted off. This may be tested in the ordinary way, and will be found to have lost much of its agglutinating power. The bacteria, if suspended in warm saline solution, will immediately clump. Evidently, therefore, the agglutinin has been removed in



combination with the bacteria. Further, it is clear that we may distinguish *two* properties of agglutinin (that of uniting with antigen and that of clumping), and that these are discharged at different temperatures: the agglutinin unites at  $0^{\circ}$ , and only exerts its specific action at higher temperatures. We may express this in Ehrlich's terminology by saying that it possesses a haptophore group which functionates at  $0^{\circ}$ , and an ergophore group which only acts in the warm.

Another proof is as follows: It was shown by Bordet that agglutination only takes place when certain salts are present. Of these sodium chloride appears to be the most generally efficient, but Crendiropoulo and Amos have shown the calcium chloride has a special adjuvant action in the agglutination of cholera vibrios. To this subject we shall return. The proof of the union between bacteria and their agglutinins is made as follows: Bacteria are added to clumping serum, and the precipitate collected and washed and shaken in a large quantity of distilled water. No agglutination occurs until salt is added, when it takes place rapidly, according to the thickness of the emulsion. In this case also the two substances must have entered into the combination.

The substance with which agglutinin combines—*i.e.*, that which calls forth its production in the living animal—is evidently not a toxin, since an agglutinating serum has, as such, no protective action. We know some of its characters. It is formed, of course, in the bodies of the bacteria, and in young cultures is entirely intracellular. In older cultures, however, it diffuses out, being probably set free by a process of autolysis, and passes into solution. This is especially the case in broth cultures, and this is one of the reasons why, if liquid cultures are used in agglutination tests, they must be *young*; in agar cultures there is less diffusion of the agglutinable substance, and the need is not so great. Its presence may be proved in two ways: In the first place, this filtrate, if injected into animals, will bring about the production of agglutinin, as we should expect. In the second place, this fluid, when added to a powerful clumping serum, will cause a precipitate. This is *Kraus's reaction*, and it is a most interesting phenomenon. It is best seen when the fluid portion of broth culture of *B. typhosus* or *V. cholerae* (at least a month old and filtered through a Berkefeld filter to remove all solid particles) is added in various proportions to a strong immune serum. Under such circumstances the fluid will gradually become opalescent, or

even opaque, then granular, and finally flocculent. It presents a most extraordinary resemblance to the clumping of an ordinary culture, but a microscopic examination will show the flocculi consist of amorphous granules instead of bacteria. It has been suggested that it is due to a clumping of cilia which have passed through the filter (Nicolle), but the phenomenon has since been observed in the case of the pneumococcus (Panichi) and other non-flagellated organisms. The agglutinable substance is thermostable. It does not appear to be given off in all cases, and sometimes all attempts to get Kraus's reaction are unsuccessful.

This substance is the antigen of agglutinin, and our nomenclature would be more uniform if we were to call it agglutin and its antibody anti-agglutin, but the terms are too firmly fixed to be altered. We shall call it agglutinable substance, or agglutigen.

The fact that heated serum still agglutinates shows that alexin or complement plays no part in the process, but we have already explained how we know that the molecule of agglutinin possesses an ergophore or zymophore group. This group, as is the case with the corresponding groups of the toxins and complements, is less resistant than is the haptophore group, and is destroyed at 70° to 75° C. The substance left is called *agglutinoid*, and is analogous to toxoid and complementoid. Its existence is demonstrated thus: Heated serum (or serum which has been kept for a long time) is added to a culture of bacteria. No agglutination takes place. The bacteria are then centrifugalized off and placed in a strongly agglutinating serum, but are found not to clump. It is evident, therefore, that the bacteria have their receptors occupied by some substance which prevents the union of the agglutinin. The agglutinoid has combined with the agglutigen, and excludes the unaltered agglutinin.

In some cases at least agglutinoids, which have a stronger affinity for bacteria than has normal agglutinin, may be present. In this case, if bacteria be added to a mixture of the two substances, no agglutination occurs. The *pro-agglutinoids* (as they are termed, the expression being taken from the prototoxoids) seize on the agglutinable substance in the bacteria before the agglutinin can do so. If to this mixture more bacteria be added, more pro-agglutinoid will be taken up, until it is all exhausted, and then any fresh bacteria that are added will be clumped. This is one explanation of a phenomenon which is fairly frequently observed (if looked for) in the clinical diagnosis of typhoid fever,

and is probably a source of error often overlooked: the serum clumps at a high dilution, and not at a low one. The author has observed it three times in the last four years. Another explanation, which is probably more often the true one, is that in the low dilutions partial bacteriolysis takes place, and the partly dissolved bacteria do not clump. The reason for this conclusion is that the clumping may occur in low dilutions in the cold, when bacteriolysis does not take place. Yet other explanations have been given.

Certain non-specific substances may bring about clumping which has a close superficial resemblance to that caused by agglutinin. This was first showed by Malvoz in the case of the action of chrysoidin on *V. cholerae*. He also showed that certain stains, such as fuchsin, vesuvin, and safranin, and some anti-septics, such as formalin (in fairly large amounts), corrosive sublimate, and peroxide of hydrogen, have this action. Mineral acids also possess this property, and also certain salts. In the case of cholera vibrios, Ruffer and Crendiropoulo found calcium chloride to have a powerful action, sodium phosphate to have a very slight one. This must not be confused with the effect of salts in favouring the action of agglutinating serum.

We are now in a position to discuss the mechanism of the process. Numerous theories have been propounded. Thus Gruber thought that the external membrane of the bacterium became "sticky," so that organisms once brought into contact remained adherent. But no visible alteration of the organisms or red corpuscles can be seen. Further, it would not account for the approach of two non-motile cells, which certainly appears to take place in clumping, and would not explain why the cells or bacteria were brought into contact in the first instance. Nicolle propounded a similar theory. He, however, showed that when inert and insoluble particles, such as of talc, were suspended in old filtered cultures of typhoid bacilli (Kraus's fluid), and serum added, they appeared to clump just as typhoid bacilli did, and it is difficult to reconcile this with his theory. Dineur thought that the flagella of the bacilli might have an adhesive material deposited on them; but many non-flagellated bacteria clump, to say nothing of red corpuscles. Others have thought that Kraus's reaction is the fundamental phenomenon, and that the bacteria, etc., are entangled in it like the particles of talc in Nicolle's experiment. But no obvious precipitate can be seen in stained

films of clumped bacteria, whereas Kraus's precipitate is easily demonstrated; besides which, agglutination can be perfectly easily demonstrated in young cultures (the fluid portion of which will not precipitate with specific serum) or with carefully washed bacteria. This explanation, though ingenious, may be disregarded.

Bordet's view is undoubtedly the correct one. It explains agglutination as being due to a change in the molecular relations between the objects and the fluids which bathe it—in other words, it is practically an effect of surface tension. It takes place in many cases other than those in which it is produced by specific sera acting on bacteria, red blood-corpuscles, etc.: thus, these objects can be made to clump by the action of many aniline stains, acids, antiseptics, etc. An emulsion of clay in distilled water will remain turbid for a long time, but will rapidly clear, owing to the formation of aggregates of particles, when salt is added. This phenomenon (which explains the formation of mudbanks at the mouths of rivers, where admixture of fresh and salt water occurs) is of especial interest in view of the necessity for the presence of salts in specific agglutination. Many bacteria, especially the tubercle bacillus, clump spontaneously without the addition of serum. In some cases this can be avoided by using a fluid poor in or free from salt to make the dilution, as in Sir Almroth Wright's method of estimating the agglutinating power of the serum on the tubercle bacillus. A process fundamentally similar can be seen if wooden matches smeared with grease are thrown on to the surface of water, and may also be seen in the gathering together of bubbles on the top of any fluid.

Two phenomena are involved: the approach of the particles the one to the other, and their adhesion subsequently. The former depends on certain physical laws investigated by Korn and others, and not yet fully elaborated, in virtue of which two elastic particles suspended in an inelastic fluid in which vibrations are taking place tend to approach one another. It is probably fair to assume that these conditions are always present in the case of bacteria suspended in a fluid medium, and that, even in the absence of any agglutinin, the individual organisms will tend to approach one another and to form aggregates. But in the case of most organisms the aggregates thus formed are quite instable, breaking up when the slightest shaking of the fluid takes place. Here the force of surface tension is all-important. It is a force

which is generated wherever a fluid comes into contact with any other substance, whether solid, liquid, or gas, and which acts exactly as if the surface of the fluid in question were in a state of tension, like a stretched film of indiarubber. If a relatively small amount of any fluid be suspended in another fluid of the same specific gravity with which it does not mix, it will assume the form of a sphere: this is because the sphere has a smaller surface for a given volume than any other solid body, and the hypothetical film on the surface continually contracts until this figure is assumed. Hence leucocytes, and most other free cells consisting of fluid or semi-fluid protoplasm, tend to assume a spherical form when in a resting condition; hence also, of course, the spherical form of soap-bubbles, oil-globules, etc. Now consider the case of two spheres acted on by surface tension and just touching one another; for example, take two drops of oil suspended in a fluid of about the same specific gravity. If we regard the surface of the two spheres as continuous, it is obvious that it is much larger than it would be if the two drops coalesced to form a single sphere. (It is roughly larger in the proportion of 4:3.) The film, therefore, will contract until the two globules are drawn into a single drop, with double the volume of each original globule, but with a much smaller superficies than that of the two separately. This process will take place whenever two bodies, neither or both of which are wetted by the fluid, are brought in contact or very close together: when one is wetted and the other not, they tend to repel one another. The force of surface tension only extends for an exceedingly minute distance into the fluid from the surface, and therefore does not draw the substances together if they are a finite distance apart. Its action comes into play when the two bodies touch one another in one point, so that the surfaces between the two bodies and the fluid join to become one at this point. Thus, if two red blood-corpuscles touch one another obliquely at one point, they become drawn together, and slide the one on the other until they oppose as small a surface as possible to the surrounding fluid. This, of course, is when the one lies flat on the other, as in rouleaux formation. Two wooden discs enclosed in a small indiarubber bag would act precisely similarly.

The exact way in which the agglutinin affects the surface tension between the bacteria and the fluid in which they lie is not quite clear, and raises difficult questions in molecular physics, some

of which are glanced at in our section on Colloidal Chemistry. It is intimately concerned with the subject of solubility. If a body is soluble in a fluid—*i.e.*, if the molecules of the latter have a greater affinity for those of the former than these have for one another—there will be no sharp line of demarcation between the two: between the solid and the liquid there will be a zone in which molecules of both substances are present, and this will shade gradually off into the solid body on the one hand and the fluid on the other. Here, then, there will be practically no surface between the two, and surface tension will be small or absent; and in a general way substances present in a fluid which dissolves them have no tendency to clump. Thus to prepare an emulsion of an oil, a solution of a soap or of an alkali is used, and the emulsions thus formed are comparatively stable; but if the fluid be made acid, the surface tension is increased, and the globules quickly run together or clump. Now it is clear from the fact that the fluid part of bacterial emulsions will give Kraus's reaction, and will lead to the production of antibodies on injection, that a certain amount of solution does take place. That agglutinin actually renders the bacteria less soluble appears clear from the phenomena of Kraus's reaction, though here the insoluble precipitate is formed on and in the bacteria, rather than in the fluid. And the complete absence of clumping which occurs when bacteriolysis takes place (though there is a large amount of agglutinin in the serum used) is an indication of what takes place when the bacteria are rendered more soluble, instead of less, by means of an antibody. Insolubility does not account for the whole of the phenomena, but it is a feature of great importance.

As regards the nature of agglutinin, all we know is that it is precipitated with the globulins, and may be of that nature. It does not dialyze, and is digested by trypsin, etc.

It appears to be formed in the lymphoid organs, red marrow, and spleen, being found early in those organs after injections of cholera vibrios (Pfeiffer and Marx). Metchnikoff found that the peritoneal exudate might be richer in agglutinins than the blood, and thought they came from the cells (leucocytes and endothelial) in that fluid. The subject has also been investigated by Van Emden, Deutsch, and Ruffer and Crendiropoulo, who all confirm Pfeiffer and Marx as to the early presence of these substances in the lymphoid tissues after inoculation.

So far the study of the agglutinins has not presented much

difficulty, but further research has shown it to be full of complexities. We will glance briefly at some more recent researches on the subject, the exact explanation and significance of which are not ascertained beyond dispute.

Several facts go to show that the agglutinin of *B. typhosus* is not a simple substance, but that two or more bodies are concerned. (It may be mentioned that this bacillus has been studied more than any other in this connection.) Thus Joos, after a series of ingenious researches, came to the conclusion that the bacillus contains two agglutinogens, and that each has its corresponding agglutinin. The agglutinin which is present in largest amount (and which he calls  $\alpha$ ) is thermolabile, being destroyed at  $62^{\circ}\text{C}$ ., leaving only agglutinin  $\beta$ , which is thermostable. An animal injected with living cultures will contain agglutinins ( $\alpha$  and  $\beta$ ) against both the substances. The first will combine with agglutinin  $\alpha$  only, whilst the second will combine with both substances. The two substances differ in their thermostability:  $\alpha$  is thermostable, but  $\beta$  loses its power of agglutination at  $62^{\circ}\text{C}$ . A couple of examples of the facts which this complicated theory was introduced to explain may be given. The serum of a horse treated with living typhoid bacilli (and therefore containing agglutinins  $\alpha$  and  $\beta$ ) clumped a living culture at 1 : 20,000, and a heated one at 1 : 1,000. When the supernatant fluid of this last dilution was tested with heated typhoid bacilli, no agglutination took place (agglutinin  $\beta$  had been removed), whereas it would clump living bacilli readily enough. Agglutinin  $\alpha$  was present in larger amount than  $\beta$ , and had not all been removed at this dilution. Again, when heated serum is added to heated bacilli there is no agglutination, since the thermolabile agglutinin  $\beta$  is destroyed. The agglutinin  $\alpha$ , it is true, is not destroyed, but its agglutinin (which is thermolabile) is. But when living bacilli are now added clumping occurs, since the agglutinin  $\alpha$  can find unaltered agglutininogen  $\alpha$  to affect.

Smith and Reagh (and their researches have been, in the main, corroborated by others) found that typhoid bacilli and other flagellated bacilli might form two agglutinins—the one acting on the agglutininogen of the bodies of the bacilli, the other on that of the flagella. The subject has also been investigated in a somewhat similar way by Buxton and Torrey, who find also two agglutinins—the one to a substance which remains attached to the body of the bacillus, whilst the other can be separated from it by

a temperature of  $72^{\circ}$ , followed by filtration. The action of the two is specific. If the filtrate be injected into animals, the serum which results clumps ordinary typhoid bacilli well, but has little action on those from which the separable substance has been removed. The serum obtained by injection of the bacilli deprived of separable substance is weaker, and has an equal action on the bacilli whether normal or heated and deprived of soluble substance.

It is evident that the subject is a complicated one, and this is even more clear from the researches of Dreyer and Jex-Blake on the agglutination of *B. coli* by its specific serum. Investigating first the behaviour of the bacilli when heated, they found, as other observers had done, no alteration at  $60^{\circ}$  C., but a sudden diminution in the power of undergoing agglutination when heated to  $70^{\circ}$  C. This, of course, is usually ascribed to the complete or partial destruction of the agglutinin, though this explanation is incomplete, since (as Eisenberg and Volk had previously found) the bacilli which do not clump will still combine with agglutinin.

But Dreyer and Jex-Blake found that the agglutinability is partially or completely restored by *prolonged* heating to  $100^{\circ}$  C. After exposure to this temperature for a period of from two to thirteen hours, the susceptibility of the bacilli to the serum might be as great, or almost as great, as at first. This is an extraordinary fact, and one for which no adequate explanation is forthcoming. The only parallel is the behaviour of megatheriolysin (a bacterial hæmolysin), also investigated by Dreyer. This is destroyed, or at least rendered inert, at  $60^{\circ}$  C., and reactivated at the boiling-point.

These authors also adduce evidence to show that the "zones of inhibition," or "pro-zones," described by Eisenberg and Volk as occurring with heated serum, cannot be explained by the assumption of the presence of "agglutinoids" with a high affinity for agglutinin. The evidence against this view is briefly this: If it were true, the more the serum were weakened by the heat (*i.e.*, the greater the production of agglutinoid), the larger should be the zone of inhibition, and *vice versa*. This they found not to be the case, for a serum which had not been appreciably injured by the heat might have a large zone of inhibition. They also found exactly similar zones in investigating agglutination caused by means of acids, in which case, of course, nothing of the nature of agglutinoids could occur. Thus, in a series of experiments it was found that when orthophosphoric acid was added to a definite



volume of emulsion of *B. coli*, agglutination occurred when between 118 centigrammes and 4 centigrammes of the acid was present, and when between 1.1 milligrammes and 0.001 milligramme, but not with intermediate amounts.

Similar phenomena (*i.e.*, the presence of zones of inhibition) can be seen in many of the actions of coagulants on colloid emulsions or solutions, such, for instance, as the precipitation of gum mastic from water by ferric chloride or trisulphide of arsenic. This case also, as Dreyer points out, shows a marked analogy with the clumping of coli bacilli by phosphoric acid, since in each case the zone of inhibition becomes smaller when the agglutinating substance (bacilli or particles of gum) become more numerous. This analogy between the agglutination of bacteria and the flocculation of colloids has been investigated by Bechhold, Neisser and Friedemann, and Biltz, and is a subject of the utmost interest, and one which bids fair to revolutionize our views on the interrelations of the antigens and antibodies. At present our knowledge of the subject is hardly sufficiently advanced to justify an account of the experiments and theories on which these views are based, and the original papers must be consulted for further information.

To revert again to the question of the specificity of the reaction : The subject is a complex one, and we have already seen that the phenomenon of the "group reaction" leads us to the supposition that bacteria of different species must possess molecules of the same nature. Further study shows that there are differences in this respect between bacteria of the same species, which are indistinguishable the one from the other by ordinary morphological and chemical tests, but which have had different origins. Thus it is found that if the serum of an animal which is strongly immunized to a given culture of *V. cholera* be tested against cultures of various stocks, that which was used for the injections will be clumped most powerfully, the others to variable degrees. These apparent anomalies, though inconvenient in practice, tend to make us regard the reaction as more specific rather than as less—sharply specific, that is, as regards a certain sort of chemical substance which is formed in greater degree by certain races of a given species than by others.

This modified specificity, sharp as regards chemical substances, but not as regards bacterial species, is well illustrated in Castellani's absorption reaction. If an agglutinating serum which, *e.g.*, clumps the typhoid bacillus powerfully, and a colon less

strongly (and which was obtained by immunizing an animal against typhoid) be saturated with typhoid bacilli, centrifugalized, and retested, it will be found that when the serum has lost its power of agglutinating that organism, it will also be bereft of power over the *B. coli*. On the other hand, if it be saturated with colon bacilli until all its agglutinating power on that organism is removed, its action on *B. typhosus* will be intact or but slightly reduced. The mechanism by which this is brought about is readily understandable by means of the following diagram (Bolduan).

Fig. 1 represents a typhoid bacillus, shown as if it consisted of three varieties of proteid material, A, B, and C, of which A is present in largest amount. The agglutinin formed by its injection will consist of three substances, each specific for its own proteid, and the antibody to A (which we may call the main agglutinin)

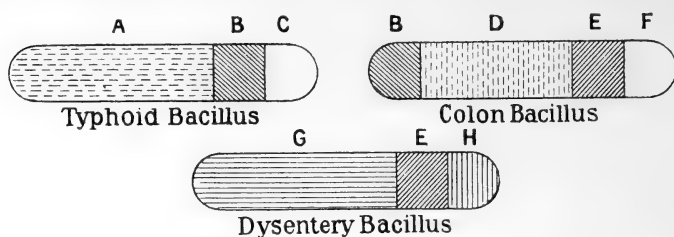


FIG. 45. (After Bolduan.)

will be most abundant. Fig. 2 represents a colon bacillus, and is shown as consisting of four forms of proteid, of which B is common both to it and to the typhoid bacillus. It follows that the antityphoid serum will clump this bacillus as well as that of typhoid, though not in so high a dilution; the serum only acts in virtue of its anti-B agglutinin, of which it possesses but a small amount, and this can only act on proteid B, which forms but a small part of the colon bacillus.

If the serum in question be saturated with typhoid bacilli the whole of the agglutinins are removed in combination with the bacilli, anti-B amongst them, and the serum will become devoid of agglutinating action on both bacilli.

But if it be saturated with colon bacilli, the only effect will be to withdraw the anti-B agglutinin; the agglutinins to A and C will remain, and consequently the serum will only lose its clumping power on the typhoid bacillus to a slight extent. After

saturating the serum with all the allied bacteria on which it can act, we might—theoretically, at least—remove all the partial agglutinins, leaving only the main agglutinin, which acts on the proteid formed by the typhoid bacillus, and by it only, and so obtain a truly specific clumping serum.

Castellani's test seems to be generally correct, though exceptions have been recorded.

Further, bacilli of the same stock can be made to vary greatly in their sensitiveness to the action of agglutinin by various methods. These have been carefully studied by Bordet, Nicolle, Kirschbruch, and others, and it has been found that the sensitiveness of typhoid bacilli is diminished by washing, by culture at high temperatures ( $40^{\circ}$  C.) or low temperatures, by the addition to the medium of minute traces of antiseptics, and is less in old cultures and in those that have been recently isolated from the living animal. It is found in general that bacilli just isolated from a typhoid patient clump badly, and that they gradually increase in sensitiveness for six months on cultivation in artificial media: a very faintly acid medium is the most suitable. Sometimes a culture alters very rapidly without obvious cause, and this is a possible source of error in the clinical application of Widal's reaction. The author in one case found a culture which was clumped by a certain serum at 1 : 60 was clumped by the same serum at 1 : 250 three days later.

Another method by which bacteria can be made to diminish in their sensitiveness to clumping is by cultivation in specific immune serum. This was first observed by Ainley Walker, and since it is of some theoretical interest in connection with Welch's theory of the nature of the unknown toxins, requires a short description. He found that when typhoid bacilli were grown in immune serum (of course, devoid of complement) diluted with broth, they gradually lost their agglutinability, and became more virulent. Thus both of the effects of "passage" were reproduced *in vitro*, and Walker further found that bacilli thus treated removed the agglutinin from the serum in which they were grown, and believed his results could be best explained on the supposition that the bacilli produced specific anti-agglutinins. This, of course, tends to corroborate Welch's interesting suggestion that some of the organisms for which no exotoxins have been discovered exert their lethal action by producing antibodies against the blood of their host.

The subject has been investigated by others, and their results do not altogether corroborate those of Walker. Thus Müller found the same inagglutinability after culture in diluted typhoid serum, but did not find any evidence of the formation of an anti-agglutinin; on the contrary, he found that bacilli thus treated had less power of weakening the action of typhoid serum than had normal bacilli. We might explain his results by saying the bacilli had lost their agglutinable receptors, or agglutino-gen. Bail, working with a different method, obtained comparable results, though he explained them quite differently. He, as well as Landsteiner and others, noticed that bacilli grown in the immune serum grew into long branching threads, losing their bacillary character entirely. This has been thought to be a sort of end-to-end agglutination.

The change is a more or less permanent one. Thus Park and Collins found that cultures (of *B. coli* and *B. dysenteriae*) which had lost their power to agglutinate might require to be grown for months on ordinary agar before they retained their normal sensitiveness. The change is evidently a very profound one, and one that is handed on for many generations.

The subject has recently been carefully investigated by Marshall and Knox, working with *B. dysenteriae*. They found the same loss of agglutinability when the bacillus was grown on immune sera, but the same change also occurred when normal horse serum was used; and they further showed that the alteration was a rapid rather than a gradual one, as Walker had found in the case of *B. typhosus*. With regard to the mechanism of the process, they proved conclusively that the modified bacilli had no power of uniting with the agglutinin, as Müller had also found. It is quite clear, therefore, that the loss of agglutinability is due to the loss of appropriate receptors, in this case at least. They point out, apropos of Welch's hypothesis of toxins, that a bacterium which attempted to protect itself against its host by the formation of antibodies would have but a poor chance of surviving, whereas by the simple process of losing some of its receptors they can nullify some of the protective mechanisms possessed by the latter.

Their explanation of the process by which these resistant bacilli are produced is interesting and suggestive. They do not think, with Walker, that the bacilli acquire a new character—*i.e.*, the power of producing antibodies—and transmit it to their descendants, but that there is simply a process of natural selection.

In any culture of bacteria it will be found that some individuals are more easily agglutinated than others. In the early cultures in clumping serum the susceptible forms grow, but they sink to the bottom in a dense network. The less sensitive forms also grow from slight granules or a diffuse turbidity through the fluid. If the subcultures are inoculated from this fluid, the change to the non-clumping form occurs more quickly than if the masses were used for the transfer. There is therefore a process of selection of the non-clumping forms, and in time all the susceptible bacilli become eliminated. It is this process, though in a more marked form, that accounts for the increased virulence of the bacteria, which is brought about by passage. For here all bacteria which are not resistant to the bacteriolysins and to the phagocytic action of the leucocytes will be killed off, and will not propagate their species. We may therefore conclude on theoretical grounds that virulent bacilli are those having (along with a potent toxin) few receptors which can be attacked by amboceptor and opsonin. We have seen that Pfeiffer and Friedberger's experiments do not tend to corroborate this (in the case of amboceptor), but that they cannot be regarded as conclusive.

The increase in the virulence of the organism by cultivation in immune serum has been generally corroborated, though Roger found the opposite to occur when streptococci were cultivated in antistreptococcic serum.

The *hæmagglutinins* occur in normal and in immunized animals. Those in the latter call for no especial notice; they are developed after injection of red corpuscles side by side with immune body, and their presence can be demonstrated if the serum be heated or if the experiment be performed in the cold. The normal agglutinins which one species may possess for the red corpuscles of another species call for no special notice.

The iso-agglutinins are, however, worthy of a short description. They are common, and have been most studied in human blood, and will be found to occur to a greater or less extent in most specimens of human sera. The phenomena they produce are entirely similar to those produced by a specific serum in a typhoid culture. Under the microscope the corpuscles will be seen to run together into masses which are quite unlike the ordinary rouleaux of shed blood in that the corpuscles are approximated together without any trace of definite arrangement. When a strong serum is used the cohesive force may be so great that the corpuscles

become absolutely fused together into a solid mass, in the centre of which the outlines of the original corpuscles cannot be made out. The macroscopic appearances are similar to those of a Widal's reaction, the emulsion of corpuscles becoming granular and flocculent, and clear rapidly, with the deposition of a red mass at the bottom of the vessel. With a powerful serum a column of emulsion 2 inches high may be completely cleared in five minutes, and the clumping, as observed in a drop of the fluid on a slide, may be complete in a few seconds.

It will be found that a certain serum does not clump all human corpuscles equally well, and facts have been observed quite comparable to those found by Ehrlich in the case of isohæmolysin. According to Landsteiner (whose researches have been corroborated by Hektoen), human bloods may be divided into three groups, which Hektoen describes as follows :

*Group 1.*—Here the *corpuscles* are not agglutinated by sera of the other groups, whilst the *sera* agglutinate the corpuscles of both groups.

*Group 2.*—In this group the *corpuscles* are agglutinated by the sera of the other groups, whilst the *sera* agglutinate the corpuscles of Group 3, but not of Group 1.

*Group 3.*—The *corpuscles* are agglutinated by all other sera, and the *sera* agglutinate the corpuscles of Group 2, but not of Group 1.

A few specimens of blood are found which do not fit exactly into any of these categories.

It will be noted that in none of these groups is there an agglutination of red corpuscles by its own serum—*i.e.*, an example of the presence of an auto-agglutinin. This substance, however, may occur in the blood, but apparently only in disease. The best example (in fact, the only one in which it has been positively demonstrated) is in pernicious anæmia. In this disease it often happens that the washed corpuscles are immediately and strongly clumped by the serum of the same patient, and the same phenomenon may occur when citrated plasma is used instead of serum. Whether similar phenomena occur in the body, and if not, the reason for its absence, is quite unknown : it is exceedingly difficult to imagine that clumps similar to what we see *in vitro* can be formed in the circulation, for they appear to be much too large to pass through the capillaries. The only plausible explanation is that the auto-agglutinin does not exist as such in the

circulation, only coming into existence as the blood is shed. If this is the case, it appears clear that its production is not the result of clotting, since the clumping may occur before coagulation has taken place; indeed, if the blood is carefully watched as it flows from a skin puncture in a marked case of pernicious anæmia, it may be seen to become "streaky," pale and dark areas being present, and a microscopical examination will show that this is due to clumping of the corpuscles, which thus appears to take place immediately the blood leaves the vessels.

The researches of Gay would appear to show that the clumping of the red corpuscles by serum is not necessarily due to the presence of an agglutinin at all, but may be caused by variations in the tonicity of the corpuscles and serum. Thus he found that in the bloods which have non-agglutinable corpuscles there is a higher molecular concentration than in the other groups, indicating a greater amount of salts both in the corpuscles and in the sera. There are also differences in tonicity, though less marked, between the members of the other groups. He also finds that if a serum which is hypertonic to a certain sample of corpuscles, and which therefore clumps them, is examined after contact with the corpuscles in question, its tonicity is decreased until it reaches that of the serum which normally accompanies those corpuscles, which, of course, it now no longer agglutinates. This is a simple saturation experiment, the old explanation of which would have been that the agglutinin had all been removed by contact with the corpuscles, but which Gay explains by absorption of salts by the corpuscles. Lastly, a simple hypertonic solution of NaCl and  $\text{CaCl}_2$ , and according to Peskind a large number of acids and acid salts, gave rise to appearances suggestive of clumping, though not identical therewith. His researches are highly suggestive, and it may be that we shall have to modify our ideas of the normal iso-agglutinins; but the subject requires further investigation.

Rouleaux formation<sup>1</sup> presents some resemblances and also some differences. Red blood-corpuscles, when washed free from serum and suspended in normal saline solution, lie free side by side, and show no tendency to run together; but if placed in human serum and some other fluids, the biconcave discs approach one another in a peculiar orderly fashion, so as to form adherent rolls resembling

<sup>1</sup> I am indebted to some unpublished researches of Dr. Wiltshire's for much interesting information on this subject. Any new fact mentioned is owing to him, unless the contrary is stated.

piles of money. This phenomenon has attracted a good deal of attention, though less than it deserves, and the exact mechanism by which it is produced is still uncertain. It has obviously some resemblance to agglutination. It is quite conceivable, for example, that an agglutinating serum, when greatly diluted, might act so weakly that the corpuscles might be drawn gradually together, and so approach one another in such a fashion as to form the peculiar rolls, very much as in the orderly deposition of molecules to form a crystal, which occurs when a strong solution of a salt is slowly evaporated, so as to allow the molecular attractions to come into play in a regular fashion. But this is not the case, since, as was shown by Descatello and Sturli, it is not possible to dilute an agglutinating serum with normal saline solution so as to convert it into a rouleaux-inducing one. And according to Lange, an agglutinating serum which has had its agglutinin removed by saturation with corpuscles will still give rise to the phenomenon under discussion.

Both the corpuscles and the fluid in which they are suspended require consideration in the discussion of the question why rouleaux formation occurs in shed blood, though not in the tissues. Human serum will always cause it, though its potency in this respect varies greatly from time to time in the same person, and also in different individuals (as tested on the same specimen of red corpuscles). This power is quickly destroyed on dilution, being almost always annulled on the addition of an equal quantity of normal saline solution. The roulogenous principle also occurs in human milk, but not—or not commonly—in that of cows, and is not destroyed by boiling for five minutes. Viscosity appears to play some part of secondary importance, and not clearly defined: solutions of colloids, such as gum or gelatin, may induce it, as was pointed out by Wharton Jones. The roulogenous substance occurs to a very large extent in inflammatory exudates, but not in transudates.

It is possible that the induction of rouleaux formation may be due to the development of some substance in the serum at the moment the blood is shed, and it is also possible that it may be due to some change in the red corpuscles. It has been shown that biconcave discs suspended in water and smeared with grease will run together in this way, and Brunton has suggested that when the blood is shed some fatty acid is liberated by the action of carbon dioxide. It is also possible that the change which



occurs when the blood is shed may be one of shape. Thus Weidenreich and others hold that red corpuscles are normally bell-shaped, and that the moment they leave the vessels they become biconcave discs. If there is an increase in the tension generated at their surface of contact with the serum, the conditions for rouleaux formation would appear to be present. Weidenreich's facts have been strongly disputed, and are not generally accepted.

Rouleaux formation cannot be induced in corpuscles which have been heated to  $44^{\circ}\text{C}$ .

## CHAPTER IX

### THE PRECIPITINS

AGGLUTININS are antibodies obtained by the injection of particulate substances, and have the property of causing these substances to collect into clumps. In exactly the same way proteids in solution, when injected into suitable animals, bring about the formation of another class of antibodies, which possess the power of clumping the molecules of the proteid in a solution similar to that injected. This manifests itself by the formation of a precipitate. After the addition of the clear antiserum to the clear proteid solution, the mixture becomes opalescent, and then opaque, and after a time a precipitate is cast down, leaving a clear supernatant fluid. Hence these substances are called *precipitins*, the substance with which they form a precipitate, and which calls them into existence when injected into a suitable animal (their antigen), being termed precipitable substance, or precipitogen, and the insoluble combination of the two, precipitum. They are in all respects closely allied to agglutinins, if not absolutely identical. The fact of their acting in a clear fluid does not prove that they exert their effect in a true solution, since modern physico-chemical research has shown that proteids do not form solutions, but merely emulsions or suspensions of molecules or of complexes of molecules. The effect of the addition of a precipitin is to cause an agglutination of these molecules, which is entirely analogous with the agglutination of typhoid bacilli. The laws which govern the reactions of the precipitins and agglutinins are entirely similar, and, theoretically, it would probably be more accurate to consider them under one head. The practical applications of the two classes of antibodies are, however, very different, and it is more convenient to treat them as separate substances.

The first substances of this group to be discovered were the bacterio-precipitins of Kraus, first investigated in 1897, and referred to elsewhere. Kraus found that, if he took an old

culture of typhoid bacilli and filtered it to remove the bodies of the bacteria, and then added some typhoid serum to the clear solution, a precipitate was formed. The same happened with cultures of cholera and plague organisms, after addition of the appropriate sera, so that the reaction is, within limits, specific. The limits of the specificity are not yet thoroughly ascertained, but there are distinct evidences of group reactions similar to those seen in the agglutinins. Thus Norris found a common precipitin for organisms of the cholera group, for some cocci, etc. There were, however, some exceptions; thus, a rabbit which had been injected with cultures of *B. prodigiosus* developed a precipitin which acted on filtered cultures of *B. coli* and *V. Metchnikovi*, as well as on the filtrate from the organism injected. He thought, however, that the reaction is a more intimate and constant test of group relationships than is agglutination; thus he prepared an antiserum to a bacillus belonging to the hog-cholera group which did not agglutinate typhoid or colon bacilli, but which gave the precipitin reaction with their filtrates. The bacillus, it may be pointed out, is a member of the same group as the other two, so that (in the case of this particular serum) the agglutination reaction is misleading.

Bacterio-precipitins may be prepared by the injection of cultures of the bacteria, or the filtrates from the old cultures spoken of above; it is evident, therefore, that they are antibodies to substances in solution. Some authorities, it is true, have thought that the actual antigen and precipitable substance consist in reality of the broken-off flagellæ, which are sufficiently fine to pass through a Berkefeld filter; but this can hardly be the case, since a bacterio-precipitin can be obtained for non-flagellate organisms. It is true that the best-known and most powerful of these antibodies are for organisms which possess flagellæ, and it is quite probable that the clumping of these filaments does occur in some cases, and intensifies, or may be mistaken for, a true precipitation.

Bacterio-precipitins cannot be (or have not been) prepared to all organisms. Thus, diphtheria antitoxin does not form a precipitate with diphtheria toxin, a substance prepared on lines exactly similar to those used in procuring the precipitating solution for typhoid and cholera. Diphtheria toxin is an excreted substance, not a substance dissolved out of the bacterial protoplasm.

The first to observe precipitins to other proteid solutions, and in particular to serum, was apparently Tchistovitch, in 1898. He

investigated the formation of an antitoxin for eel serum by injecting that substance into rabbits, and found the serum thus obtained not only neutralized the toxic effects of the eel serum, but formed a precipitate with it. In an exactly similar way, he prepared a precipitating serum to a non-toxic serum—that of the horse—and investigated its properties. He found that the precipitate formed by the interaction of the antiserum and its antibody was soluble in dilute acids and alkalis, but insoluble in water, alkaline carbonates, and neutral salts. These results were corroborated by Bordet, whose classical researches on the hæmolysins were made about this time. He found that the injection of defibrinated fowl's blood into rabbits caused the appearance of agglutinins, hæmolysins, and of precipitins in the serum of the latter, so that the fowl's red corpuscles would be first clumped and then laked, after the addition of the immune serum, and this substance, when added to fowl serum, led to the formation of a precipitate. Bordet also demonstrated the formation of a precipitating serum for milk (lacto-serum). These researches were corroborated by Myers, Uhlenhuth, Wassermann and Schütze, Nuttall, and others, and the reaction has now been very fully studied, and found to be of considerable practical importance.

Precipitins are in all respects closely analogous to the agglutinins. They are formed under the same conditions—*i.e.*, as a reaction of the cells to a foreign material, probably in all cases of a proteid nature—and appear themselves to be proteids. They are destroyed by pepsin and other proteolytic enzymes, and by acids and alkalis. They appear to be allied to or carried by the globulins. When heated they appear to undergo a change into *precipitoid*, a substance which has the power of combining with the molecules of precipitogen, but which does not precipitate them; this is shown by the fact that a further addition of precipitin does not cause precipitation, indicating that the effect is not due to a mere weakening of the substance or to its partial destruction. Hence we deduce that the precipitin molecule consists of two portions—a thermostable haptophore or combining group, and a thermolabile functionating group, the action of which is necessary for agglutination of the molecules to occur. This change takes place at a temperature of 50° to 60° C., but it also occurs slowly when precipitin solutions are kept at ordinary temperatures, so that these become gradually useless as tests for their antigens; and the change may be hastened by the action of light or of various

chemical substances. Precipitating sera should, therefore, be kept in a dry state in a cool place, and preserved from light.

Precipitoids appear to have a stronger affinity for the precipitate substance than has the unaltered precipitin—an alteration in affinity similar to that which we have seen to occur sometimes in the case of agglutinin, and which leads to the formation of what we have termed pro-agglutinoid. Thus, if a serum which has been heated until it has lost its precipitating power be mixed with unheated precipitin, the mixture will not form any precipitate after the addition of small amounts of the normal serum; it is only after enough of the latter has been added to combine with all the precipitoid that a precipitate begins to appear. The same phenomena may occur in working with old and degenerated sera, or occasionally even with fresh ones. An example will make this clear.

Normal Serum.	Antiserum.	Amount of Precipitate.
7	1	nil
6	1	nil
5	1	nil
4	1	nil
3	1	nil
2	1	0.5
1	1	1
1	2	1.25
1	3	2
1	4	3
1	5	3.5
1	6	3.75
1	7	4
1	8	4
1	10	3.25
1	12	2.75
1	14	2.25
1	16	2
1	18	1.5
1	20	1
1	24	nil
1	30	nil

Here the maximum precipitate was given when 8 parts of antiserum were mixed with 1 part of normal serum. When 1 part of normal serum was mixed with 24 parts of antiserum, there was no

precipitate—*i.e.*, the precipitoids were at this point just saturated ; but in a mixture of 20 parts of antiserum with 1 part of normal serum, it appears that, after all the molecules of precipitoid were saturated, there was still enough precipitable substance present to combine with some of the precipitin, and thus to form a precipitate. In the mixture of 8 and 1 the precipitoid was all saturated, and a maximum amount of precipitable substance left over to combine with precipitin.

This theory of the "specific inhibition" of the action of this precipitin was the first explanation to be brought forward, and appears to afford a fairly satisfactory explanation of the facts observed. It is, however, quite probable that future physico-chemical research may show it to be erroneous, and that these zones of inhibition are in reality similar to those observed in the non-specific precipitation of colloids, of which we have already spoken. To this subject we shall return. Be the explanation what it may, the phenomena are of considerable practical importance in the application of the precipitating sera to the diagnosis of the nature of an unknown serum or other solution of proteid. The mere fact of not obtaining a precipitate when a solution of unknown strength (*e.g.*, of a dried blood-stain) is added to an anti-serum is not necessarily of any importance, and to obtain accurate results it is necessary to perform a series of quantitative experiments.

The experiments of Eisenberg, Michaelis, Fleischmann, and others tend to show that the combination between the two obeys the laws governing the combination of weak acids and bases, which we have already discussed, and that when the two sera are added together both free precipitin and precipitable substance may be present at the same time. This, if true, would not explain relations between the precipitin and precipitable substance similar to those given above ; if the law of the mass reaction applied, an excess of antiserum would tend to give the maximum possible quantity of precipitum, though there would always be some unaltered precipitable substance present in an unaltered state. It is, however, doubtful whether the substances do actually interact as weak acids and bases. Von Dungern, experimenting with antisera obtained to the proteids of cold-blooded animals, found that the reaction was rigorously quantitative, but that a complication was introduced by the presence of two varieties of precipitin, special and partial. The special precipitins are sup-

posed only to act on the proteid which has been used for the immunization, whereas the partial ones act both on it and on other foreign proteids. If these are formed at different rates of speed, it is easy to see that at a certain stage in the process of immunization an animal's serum may contain a precipitin (*e.g.*, the special precipitin) and a precipitable substance (*e.g.*, that which acts as an antigen to the partial precipitin). In most of their reactions the precipitins certainly act as if they had a powerful combining affinity for their antigens, and von Dungern's observations may supply the explanation of the apparent discrepancies.

If precipitable substance (*e.g.*, egg-albumin) be heated, it undergoes a change comparable to that sustained by precipitin on heating: it loses its power of becoming precipitated with antiserum, but retains its function of combining therewith. This altered proteid is sometimes called precipitoid, since it is analogous with the precipitoid derived from precipitin by the action of heat. The term, however, is a bad one, since its use leads to confusion between the two substances, and the only word which is at all suitable is "precipitogenoid." Precipitogenoid, therefore, consists of the haptophore portion of the molecule of precipitogen, another portion of this molecule which must be present in order that the reaction may take place being destroyed. From the fact that it retains its haptophore group we should expect it to act as an antigen, just as toxoid does; and this is the case, for the injection of precipitogenoid calls forth the production of ordinary precipitin. In any complete quantitative study of the interactions of serum and antiserum it is necessary to investigate the question of the presence of precipitogenoid as well as of precipitoid, and the problems thus may become complicated in the extreme.

Precipitins, like the agglutinins, act most quickly at body temperatures, and show their essential similarity in the fact that salts are necessary for both reactions; short of absolute absence, the amount of the precipitum form depends on the quantity of salts present (Friedmann).

There is an interesting analogy between the agglutinins and the precipitins, in that the latter as well as the former are occasionally seen in the serum of unimmunized animals, though the precipitins are much more uncommon in this situation. Thus, Hoke found the presence of bacterio-precipitins to filtrates from *B. typhosus* and *V. cholerae* fairly frequently present in the serum of the ox, more rarely in that of the goat, pig, and sheep. The presence of

serum precipitins is rarer, but occurs, according to Noguchi, in cold-blooded animals (crustacea, etc.), and Lamb found in normal rabbit serum a precipitin for cobra venom, and Obermayer and Pick one for dysglobulin of egg-white in the same fluid.

The relation of the precipitins as regards specificity forms a difficult and most important question. It was at first thought that the specificity was a sharp one, and that a serum prepared by the injection of human serum would only precipitate with human serum, and a lacto-serum prepared by the injection of cow's milk would only precipitate with that fluid, and not with the milk of other animals. Thus, Uhlenhuth prepared a precipitin by injecting a rabbit with ox serum, and found it gave a precipitate with that fluid, but not with the serum of the horse, donkey, pig, sheep, dog, cat, deer, fallow-deer, hare, guinea-pig, rat, mouse, rabbit, chicken, goose, turkey, or pigeon; but he also showed that anti-egg serum was not sharply specific, and would coagulate solutions of albumin from eggs other than those of the species used for the injection. Wassermann and Stern also showed that antihuman serum would react, though but slightly, with the serum of the baboon, and Stern confirmed this, and found it reacted with the serum of other monkeys. Hence the idea gradually arose that the precipitin obtained by the injection of a serum from one species of animal is not specific for that species, but will give a precipitate, though of less amount, with the sera of other species, provided that they are sufficiently closely allied zoologically. This has been especially studied by Nuttall, who expresses this relationship between species close together in the animal scale as a "blood-relationship." He showed that, provided the serum were powerful enough, it would react with all the bloods of animals in the same great division of the animal kingdom (mammalia, birds, reptiles, amphibia, etc.). Thus, a strong antihuman serum will give a precipitate with human serum, even when highly diluted, with apes, monkeys, etc., but not in such high dilutions, and a slight trace of precipitate after a long period when mixed with the sera of more remote mammalia, but no precipitate with the blood of birds, fishes, etc. Quite similar relationships hold with the lacto-sera and with the precipitating sera for muscle proteids; the anti-sera for egg proteids is apparently less specific.

Hence we deduce that the precipitins are not specific as regards the animal species from which they are derived, but possess that partial specificity seen in the cytotoxins and in the "group



reactions" of the agglutinins; that is to say, they are specific as regards the antigens which bring them into existence, irrespective of the source from which that antigen was derived. This appears to be substantiated by experiments on purified and recrystallized proteids, the precipitating sera for which show a high degree of specificity. Too frequent recrystallization of proteids, however, appears to injure their power of inducing the formation of precipitins.

Further, proteids which have been altered in character by chemical processes (iodized, nitrified, or denitrified) will cause the formation of precipitins which are specific for the transformed molecules of proteid, no matter from what source they were derived. These observations are of profound interest, since they appear to show that it is possible by artificial means to alter completely the haptophore group of a proteid molecule. According to Uhlenhuth, there is little or no specificity in the antisera prepared against the proteids of the crystalline lens. An antiserum prepared by injections of serum will precipitate all albuminous fluids except a solution of the lens, which in its turn will not precipitate with serum. But an anticrystalline serum prepared by the injection of ox lenses into rabbits will give precipitates with lens solutions from mammals, birds, amphibia, and even fish.

Hence, too, a practical point in connection with the employment of precipitating sera in the detection of the source of blood. Here it is necessary to use a high dilution of the blood to be tested, and if a reaction is given, to try again with higher dilutions until the limit is reached. In testing dried blood-stains for medico-legal purposes it is, of course, usually impossible to determine the exact amount of serum which has been taken up by the solvent (normal saline solution). It is found, however, that a 1 : 1,000 dilution of serum in normal saline solution will give a good froth when air is allowed to bubble through them from a pipette, and this will give a rough idea as to the amount of proteid material dissolved out of the clot to be tested.

The delicacy of the reaction is truly astonishing. Thus Ascoli obtained an anti-egg albumin serum which gave a precipitate with a 1 : 1,000,000 dilution of egg-albumin, and Stern an antihuman serum which reacted with serum at a dilution of 1 : 50,000. These are extreme figures, but sera active on solutions diluted 1 : 5,000 are frequently obtained.

As regards the substances for which precipitins can be obtained,

we find them limited in all cases to the proteids. All the coagulable proteids will give rise to the formation of precipitating sera. As regards the formation of these substances by means of digested proteids (peptone and albumoses), the facts are less certain. Myers obtained a precipitin to Witte's peptone, but according to Obermayer and Pick complete peptic digestion destroys both the power of inducing the formation of antibodies and of being precipitated. The former is first to go, so that a partially digested proteid solution which will no longer precipitate with an antiserum will yet lead to the production of a precipitin when injected into a suitable animal. The results with tryptic digestion appear to be entirely similar. Complete destruction leads to loss of both functions. It appears, therefore, that it is only the giant molecule of proteid that can be agglutinated; the smaller, diffusible molecule of the products of proteolysis, like the molecules of toxin, though they may, and probably do, unite with their antibodies, do not manifest this combination by forming clumps. Proteids which have been coagulated by heat have still the power of forming a precipitin (which, of course, manifests its action on solutions of the unaltered proteid) when injected.

As regards the species of animal from which the precipitins are to be prepared, it is natural to choose an animal as remote in the zoological scale as possible. In the case of human sera or indifferent substances, such as solutions of albumin, etc., the rabbit is usually chosen, since it is easily obtained and handled, and will yield a very considerable amount of serum. Where antisera to animals closely allied to rabbits are to be obtained, fowls or other large birds are most suitable. There are, however, some observations which go to show that there are differences between individual rabbits which are comparable in every respect to those between the different red corpuscles of goats and other animals, as seen in Ehrlich's experiments on the isohæmolysins. Thus Schütze injected the serum of rabbits into rabbits. In two cases out of ten he obtained a precipitin which reacted with the serum injected. This substance is called isoprecipitin. It may often be noted that a precipitate falls when different samples of diphtheria antitoxin (in animals in which the earlier stages of the process of immunization have been carried out by means of serum toxin) are mixed together, and this is probably an analogous phenomenon. These precipitins are, however, very feeble as compared with those obtained by the injection of the serum of a certain species

into an animal far removed in the zoological scale, and their explanation is obvious in the light of what has been already said with regard to individual differences in receptors.

According to Ewing, an antihuman serum prepared from the fowl shows a far higher degree of specificity than those obtained by injections into rabbits.

A short account of the practical application of the precipitins may not be out of place. The chief is, of course, the medico-legal identification of blood-stains, the chief exponents of which are Uhlenhuth and Wassermann. The antiserum is obtained from rabbits, which are treated by intravenous or intraperitoneal injections at intervals of three or four days. The material used for the injection may be blood obtained by venesection or vein puncture, or from the placenta or from the cadaver, or pleuritic or ascitic fluid may be used; in any case strict asepsis is necessary. The amount given rises from 1 to 3 or 4 c.c. in the case of intravenous injections, or twice as much or even more in the peritoneum. The course of treatment lasts three or four months. Another and simpler method is to give larger doses—up to 10 c.c., or even more—intraperitoneally at intervals of a week. The animal is then chloroformed, and as much blood as possible collected either from the heart or carotid artery.

The fluid to be tested is prepared by maceration of the clot, piece of stained linen, etc., with normal saline solution, or with 1 per cent. NaOH. In the case of a very old stain, Ziemka recommends the use of a strong solution of potassium cyanide, which is subsequently neutralized with tartaric acid. This is examined with the microscope and tested spectroscopically to determine the presence of blood-corpuscles and pigments. The solution is then filtered. Air is then allowed to bubble through the fluid to make sure that proteid material has actually passed into solution; this is indicated by the production of a stable foam. Three tests are made. In the first tube one part of the fluid under examination is mixed with two of the antiserum, the second contains the fluid alone, and the third antiserum plus normal saline solution. Further controls, in which the antiserum is mixed with diluted serum from animals other than man, may also be made if necessary. The tubes are usually incubated and examined from time to time, and a positive result is obtained if there is a precipitate in the first tube and not in the others. Further tests are then made with greater dilutions, and with a

powerful antiserum a reaction can usually be obtained in dilutions so high that proof of the presence of proteids is barely obtainable by ordinary chemical means. The weak point in the method is that it is never possible to say exactly how much of the proteid matter of the clot has been dissolved, and thus to compare the effect of the antiserum on the solution with its action on diluted serum of man and of other animals. Given a sample of unaltered and undried serum, the test can be carried out with almost complete certainty; but this is rarely, if ever, possible in actual practice.

Another test, based on Gengou's reaction, has recently been introduced by Neisser and Sachs. It is carried out in the following way: A hæmolysin for ox corpuscles is prepared by injecting these bodies into a rabbit. Another rabbit is injected with human blood, so as to lead to the production of a precipitin. When the test is to be made the fresh serum of the latter animal (or if only stale serum is at hand, some fresh normal rabbit's serum must be added to supply complement) is added to the fluid to be tested. If human serum is present, even in an amount so small that no precipitate is formed, the antigen and antibody combine and withdraw the complement from solution. To test this ox corpuscles are sensitized with the heated (decomplemented) serum of the first rabbit and thoroughly washed, and some of the mixture added. If the complement has been withdrawn, of course no hæmolysis will occur. Certain obvious controls are employed to demonstrate that the corpuscles were actually sensitized, and that complement was present in the rabbit's serum before the addition of the fluid suspected of containing human blood.

This test is extraordinarily sensitive, Neisser and Sachs finding that the millionth part of a cubic centimetre of human serum was readily demonstrable. They claim also that it is more specific than the ordinary precipitin test, it being necessary, for instance, to use  $\frac{1}{1000}$  c.c. of ape's serum to get the same result. But the technique is complicated, and it appears, moreover, that complement may be abstracted in an altogether non-specific manner by substances other than the combination of antigen and antibody. Thus Uhlenhuth examined some spots of blood on a sack which a cabdriver (who was found dead) used as a seat, and found it brought about a fixation of complement, though it gave no reaction with the precipitin test. He then tested the material of which the bag was made, and found it also had the power of absorbing

complement. According to Neisser and Sachs, however, this power is destroyed if the serum solution is boiled, but is unaltered in the case of non-specific substances. Another serious objection is that a similar deviation may be brought about by means of sweat, so that if the reaction were obtained in a stain on body-linen it would be of little value.

The precipitin reaction has also been used for determining the nature of meat (whether fresh, as in the case of beef suspected to be horse-flesh, or prepared, as in sausages, etc.). The serum is prepared by injecting meat-juice or an (unheated) watery extract of the meat, and the test is carried out on lines similar to those described above. It has also been employed to determine the nature of bones, *i.e.*, whether they are human or otherwise.

## CHAPTER X

### PHAGOCYTOSIS

METCHNIKOFF'S researches on phagocytosis in the lowly organized animals formed a starting-point for an entirely new series of researches on the subject of immunity, and his treatise on the "Comparative Pathology of Inflammation" must ever remain a great medical classic, as well as a most fascinating work. Metchnikoff was primarily a biologist, and his attention was attracted to the spectacle of amœbæ and other unicellular organisms containing bacteria. In these lowly constituted animals the process by which the cell takes in foreign particles can be watched with ease, and the steps of the process traced. The amœba throws out arm-like processes, which surround the bacterium, close on it, and in a few minutes an organism which was previously lying free is deeply embedded in the animal's protoplasm. Metchnikoff watched its fate, and found it lost its sharp outline and clear appearance, became granular, and in a little while disappeared altogether. He found also that, in many cases at least, a minute vacuole was formed round the ingested organism, and further research showed that this vacuole contained a fluid which was acid in reaction and held in solution a digestive ferment allied to pepsin. Considering this observation more closely, it is obvious, firstly, that the amœba must be regarded as being immune to the organisms which it ingests and digests, and, secondly, that in this case the processes concerned in immunity are those concerned in nutrition: the amœba is immune to the bacterium because it can make use of its protoplasm as nourishment.

But this process, as Metchnikoff soon found, is not confined to the unicellular organisms. His most beautiful and classical series of researches deal with the properties and action of the leucocytes in a small fresh-water crustacean (daphnia), which, from its transparency and small size, is a very suitable object for

observation. Leucocytes, it may be pointed out, are cells which, from their isolation from one another, power of independent movement, etc., are closely analogous with amœbæ and other lowly organized protozoa.

Metchnikoff found that daphnia is subject to a disease due to the invasion of its body-cavity by the spores of a yeast—the monospora—and that if these spores gained access in large numbers they multiplied, formed into mature organisms, and finally killed their host. When, however, *few* spores gained access, he found that the daphnia's leucocytes approached them,

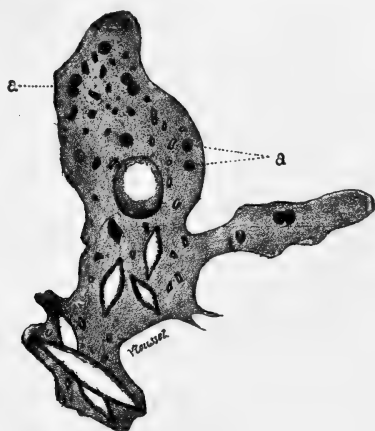


FIG. 46.—AN AMOEBA WHICH HAS INGESTED NUMEROUS SPECIMENS OF MICROSPHERA. (Metchnikoff.)

*a, a,* Vacuoles.

formed a wall round them, and finally digested and destroyed them. It is obvious, therefore, that the immunity of these animals is relegated, so to speak, to its leucocytes. If these are efficient, the animal is preserved from its invaders; whereas, if they make default, the latter multiply, and bring about the lethal issue.

Metchnikoff's experiments were by no means confined to the action of the leucocytes on bacteria. They included a careful and exhaustive study of the method of absorption of all manner of particles, organized and unorganized, in the tissues and body-cavities of animals of all positions in the animal world, and they proved to the full the importance of phagocytosis and intracellular digestion, and one of the chief—if not, indeed, the only—

method by which intruding particles are dealt with in the animal economy. And, further, they correlate in the clearest possible way this "scavenging" of the tissues with normal processes of

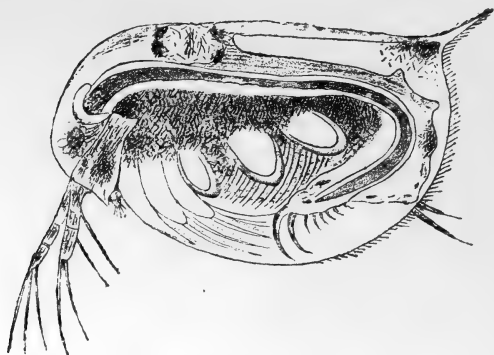


FIG. 47.—DAPHNIA CONTAINING LARGE NUMBERS OF MONOSPORÆ.  
(Metchnikoff.)

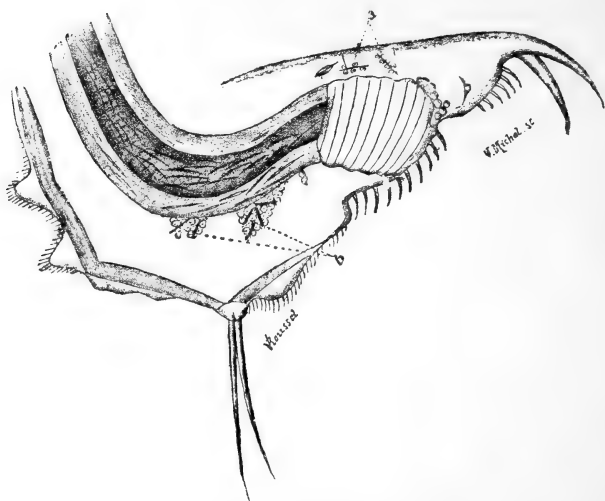


FIG. 48.—HIND PART OF DAPHNIA.

At *a* the spores of monospora are shown surrounded by leucocytes.

digestion and nutrition. Take, for instance, the absorption of the red blood-corpuscles of birds, which are very suitable for research, being readily recognized (they are nucleated), non-poisonous, and easily digested. Metchnikoff showed that these



substances are absorbed from the alimentary canal of the lower invertebrates by a process of intracellular digestion, and by that alone. Thus, when the alimentary canal of a planarian (*Dendrocaelum lacteum*, an animal resembling the liver-fluke in its general anatomy) is filled with blood, the latter is found to undergo the changes in colour familiar in bruises, and this is due to the fact that the blood has been taken from the alimentary canal by the cells lining it, and there has undergone the digestive changes. These processes can readily be traced under the microscope, and the blood-corpuscles can be seen, at first embedded in protoplasm and of normal contour, and later enclosed in vacuoles and of altered shape. Complete digestion takes several days, and every stage of the process is easy to watch. Absorption of organized food particles takes place from the alimentary canal in exactly the same way in actinians, molluscs, and many other lower animals, and in many of the cases which have been investigated the vacuoles are found to contain a digestive ferment allied to trypsin or pepsin. In higher animals this process of intracellular digestion does not occur, or only to a slight extent (in the case of fats), the animal finding it more advantageous to secrete the digestive juices into the alimentary canal, and to absorb the products of their action therefrom in a state of solution.

Absorption of particulate substances which have gained access to the tissues takes place, to a very large extent, in a method entirely similar. Thus Metchnikoff showed that when bird's corpuscles are injected into the tissues of the larva of the cockchafer, or into the snail, earthworm, the peritoneal cavity of the goldfish, etc., the process is also intracellular and entirely similar to those which occur when these corpuscles are injected into the alimentary canal of the planarian. In most cases the corpuscles are absorbed by the leucocytes, in others by the cells of the part (such as the endothelial cells lining the peritoneum); but in all cases there is the same vacuolation, the same series of changes in the ingested corpuscles, and the same final result. We shall not be far wrong in associating the absorption of these corpuscles in a very close way with processes of digestion and nutrition.

The French school have studied these processes of absorption of particulate bodies from the tissues in a very complete manner, and have shown beyond dispute the importance of phagocytosis in this respect. Thus particles of carbon in the lung, the granules of pigment left after interstitial hæmorrhages, etc., are all ingested

and removed by phagocytes of one sort or another, and this discovery throws a flood of light on the meaning of many of the phenomena of inflammation, more especially on the leucocytic invasion of the injured tissues, which has for its object, in part at least, the removal of particulate substances which are the cause of the injury. Further, the dead or injured tissues, the result of the action of the irritant, are eroded and removed by phagocytic action. If a *small* volume of tissue, situated in a region to which numerous leucocytes can gain access, be killed, it may be completely removed piecemeal in this way. We may quote as an example the complete absorption of the central slough which often takes place in acne pustules or small boils, especially after treatment with staphylococcic vaccine. Very interesting in this connection is the process of the absorption of the tail of the tadpole, which is removed in an entirely similar way by the action of phagocytes. And it is worthy of notice that the digestive power of the phagocytes is a very powerful one, and substances usually deemed entirely insoluble may be gradually removed by their action.

We have already referred to the action of leucocytes in absorbing and neutralizing toxins, and have quoted the beautiful experiments of Besredka on trisulphide of arsenic, which, when placed in the peritoneal cavity, is absorbed by these cells and prevented from exercising its poisonous action; whilst, when shielded from their attack by being enclosed in bags which are permeable to fluids, but not to solids, it undergoes gradual solution, and leads to fatal intoxication.

Metchnikoff applied these researches on phagocytosis to the question of immunity, and formulated a complete and logical theory on the subject, which he illustrated with many striking researches and examples. For him—and modern advances tend more and more to corroborate the truth of this view, though in a much more complicated way than he thought—the defence of the animal economy is entrusted entirely to the phagocytes, and especially to the leucocytes. If a bacterium enters the tissues these cells may at once make their way to the seat of infection, and proceed to ingest the bacteria and kill them intracellularly. In this case the animal recovers, with or without a transient illness, and we say it is immune. If another species of bacterium enters, the effects may be different: the leucocytes may, perhaps, be repelled instead of being attracted, or, if attracted, may be killed

by the action of the bacterial toxins, so that no phagocytosis occurs; or perhaps they may take up the bacteria and then be killed by the toxins. In any case, the result is the same: the bacteria continue to grow and to produce their toxins, and the result is death. The animal is susceptible to the bacterium because its leucocytes are unable to deal with it. Immunity, therefore, is a function of the phagocytes.

So far we have dealt with natural immunity. The application of Metchnikoff's theory to acquired immunity is equally simple, though much less satisfactory. He argues that the leucocytes, in their contest with a particular species of bacterium, become educated to overcome this bacterium, and are able to deal with it in future with great ease. In the first infection there may be a balanced contest of some severity and duration, but as a result the leucocytes, like war-trained veterans, are readily able to cope with the invader a second time. This theory, though ingenious, cannot be maintained at the present day. Its truth rests, of course, on the truth of Metchnikoff's main thesis, which is only partially true, and which is only one factor in the complicated phenomena of immunity. We may just point out, however, that the life of a leucocyte is, in all probability, a comparatively short one, to be measured by days, or at most weeks, so that acquired immunity due to the education of the leucocytes would be of short duration. Nor is it of any assistance to argue that in the struggle against the invading bacterium the fittest leucocytes would survive, and so lead to the general improvement of the leucocyte species: for leucocytes do not propagate themselves, but are emitted from the bone-marrow, run their course in the blood, degenerate, and die, their place being taken by others from the same source. The education, therefore, must be one of the bone-marrow, and we cannot conceive how this could take place as the result of phagocytosis going on in a distant area. It is possible that something of the sort may occur, but only by the action of toxins and other bacterial products circulating in the blood-stream, and being thus brought into the marrow. Other considerations might be urged, but the theory has now but a historic interest. It has served its purpose: it has been the means of suggesting many researches which have helped greatly in the elucidation of a most difficult subject.

Before discussing the rôle of phagocytosis in the infective processes we must deal briefly with two subjects: firstly, the means

by which the phagocytes are brought into contact with the bacteria—in other words, of *chemotaxis*. This is a phenomenon which is displayed by almost all motile and unicellular organisms, whether animal or vegetable, and by the leucocytes of the higher animals, and manifests itself in a movement of the organism in response to a chemical stimulus. To take an example from the bacteria: if a capillary tube containing a solution of meat-extract be placed in a watery emulsion of *B. coli* or *B. typhosus*, the bacteria will be seen to group themselves round the mouth of the tube, which they ultimately enter. This is an example of positive chemotaxis, the bacteria being attracted by the extractives, which, indeed, they utilize as food. On the other hand, bacteria will tend to remove themselves from an area from which alcohol and similar substances are diffusing: this is negative chemotaxis. As a general rule, we may say that motile organisms tend to be attracted into a region rich in useful and nutritious substances, and are repelled from injurious ones, but this is not invariably true in the artificial conditions of experiment. An organism may be lured by a useful substance into a region where there is a sufficient quantity of an injurious one to kill it.

In cases where leucocytes make their way into a tissue infected with bacteria it must be, therefore, because the latter give off a substance which has a positive chemotactic action on them. This action may readily be shown by experiment, and the easiest way is to work with the lymph of a cold-blooded animal, since in this way all difficulties connected with a warm stage are avoided. If, for instance, we take a few anthrax bacilli and place them on a slide, add a drop of frog's lymph (from the dorsal lymph sac), and apply a cover-glass, the leucocytes can be easily seen to crawl actively up to the bacilli. To this experiment (Kanthack's) we shall have to recur.

In nearly all cases we find that leucocytes are attracted in large numbers into the area in which the bacteria are situated—*i.e.*, nearly all bacteria give off substances which are positively chemotactic for leucocytes. In a few cases, however, this appears at first sight not to happen, for when tissues which are infected with very virulent bacteria are examined microscopically, they are often extremely poor in leucocytes. As an example, we may take any example of acute spreading gangrene of bacterial origin. But, as Kanthack pointed out, these are not necessarily examples of negative chemotaxis, and it is quite probable that the paucity

of the leucocytes is due to their paralysis and destruction by the powerful toxins which are given off. Certainly since the use of methods involving experiments on phagocytosis *in vitro* no valid example of negative chemotaxis has been adduced, and it is highly probable that leucocytes are attracted by all bacteria, whether their toxins are mild or potent. In the former case the leucocytic infiltration will be very obvious; in the latter the cells will be first paralyzed and then destroyed as soon as they reach an area in which the toxin is present in a high degree of concentration.

We must not assume that this property of being attracted by bacteria is of any advantage to the leucocyte itself, arguing from the fact that the free-swimming unicellular organisms are attracted by food and oxygen. The leucocytes are in many cases attracted into the infected area to their own undoing, and it must not be forgotten that even in an inflammatory process which is mild in nature and favourable in result the number of leucocytes which may be killed in the conflict is enormous. The leucocytes are not independent protozoa inhabiting the blood and tissues, but an integral part of the organism. It is to the advantage of the latter that the former should be attracted at once to the seat of invasion, and hence the processes of evolution have led to the development of this function in the nomadic cells of the body. These are extraordinarily susceptible to chemotactic influences; they seem to be attracted by *any* deviation from the normal constitution of the tissues and fluid: a slight injury, a hæmorrhage, the presence of a poison, of a foreign body of any sort or of any dead or useless tissue, and the leucocytes are immediately attracted into the area affected. The more we regard the process, the more we must regard it as one of the most exquisite examples of means to ends met with in the animal economy.

Secondly, with regard to the nature of the cells which have the power of acting as phagocytes. Of these the most important are the leucocytes, and especially the polynuclear and large hyaline cells of human blood. *All* the leucocytes, however, have phagocytic powers, as is well seen in opsonic estimations: the cells which take up the fewest bacteria are the eosinophiles and the small lymphocytes. The former are very deficient in this direction, and we may be certain that their main function is entirely different. The lymphocytes, too, take up but a small number of bacteria; but when activated by a suitable hæmopsonic serum, they take up red blood-corpuscles in considerable numbers, and a

lymphocyte may then present a remarkable appearance, having ingested two or even three red corpuscles, each as large as itself, the narrow band of protoplasm being extended over the corpuscle in a most extraordinary way.

It is noticeable, too, that even among the polynuclears there are great differences in phagocytic powers. This is best seen in a film of pus from a case of gonorrhœa, in which certain cells (indistinguishable from the others) are packed full of cocci, whereas the vast majority are entirely free. The same fact is brought out

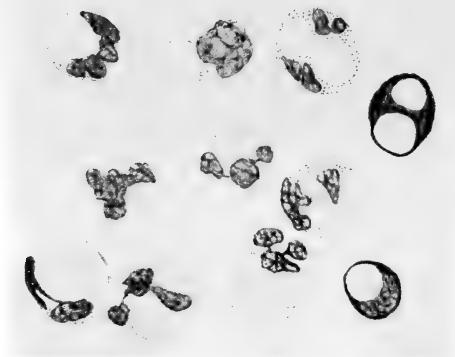


FIG. 49.—RED CORPUSCLES INGESTED BY POLYNUCLEAR LEUCOCYTES AND LYMPHOCYTES. (Original.)

clearly in opsonic experiments, where some leucocytes are often found to take up very large numbers of bacteria, the general average of the other cells being low.

Besides the leucocytes, some of the tissue cells, which are either free or have the power of becoming so, are active phagocytes. Of these, the most important are the endothelial cells. These are only flat plates of protoplasm when under normal conditions. When submitted to the action of almost any irritant they become cuboidal or columnar, and are then most active phagocytes. A good example of this may sometimes be seen in sections of a thrombosed vein at a certain stage: the endothelial cells are columnar and contain much protoplasm, and this latter is packed with pigment granules absorbed from the altered blood in the lumen. But the process goes farther than this, and the endothelial cell (whether of the serous membranes, vessels, or lymph clefts) either breaks loose from its attachments or buds off

fresh cells, in either case leading to the production of free and motile endothelial cells, which have the closest resemblance to the large hyaline cells of the blood, though they may be much larger. These cells are most active and important phagocytes, especially in the peritoneum. They have also the power of undergoing organization, especially into fibrous tissue, and many, if not all, of the fibroblasts of granulation tissue are endothelial in origin. Further, some at least of the giant cells so familiar in chronic inflammatory processes are derived from the endothelial cells of the lymph clefts and lymph capillaries. This has been proved to demonstration by Bergengrün in the case of the giant cells in leprosy. Our knowledge of the origin of the giant cells of tubercle is less exact, but analogy with those of leprosy would lead us to infer that they are endothelial also. In both diseases the giant cells are most important phagocytes.

Epithelial cells only exceptionally act as phagocytes. We have already referred to the ingestion of fat by the columnar cells of the intestine, and the other important example is supplied by the epithelial cells lining the alveoli of the lungs. These are flattened plaques under normal conditions, but in the presence of an irritant they become cuboidal or columnar, detach themselves, or bud off similar cells, and are powerful phagocytes. These are the dust cells so frequently seen in sputum.

The nature of the cells which take part in phagocytosis is determined to some extent by the nature of the irritant. Thus, when the pyogenic bacteria are ingested it is usually by polynuclear leucocytes, whereas it is extremely rare to find these cells containing tubercle bacilli in the tissues, though they will take them up readily enough under the artificial conditions of opsonic experiments. Metchnikoff classifies phagocytes into two groups—macrophages and microphages. His description of these cells is not absolutely clear, but in general the microphages correspond to the polynuclear leucocytes, and the macrophages to the large hyaline cells of the blood and the endothelial cells of the serous sacs and connective tissues. He claims that the former are especially concerned with the phagocytosis of bacteria, the latter with red blood-corpuscles and similar objects. This distinction is not a valid one, since endothelial cells are extremely active phagocytes for bacteria, and polynuclear cells will ingest red corpuscles with great readiness when provided with a suitable opsonin (see Fig. 49). It would appear that under

suitable conditions any phagocyte can ingest any abnormal body of suitable size.

Under certain conditions there may be traced a remarkable sequence in the advent of various phagocytes to an infected area, which almost suggests a symbiosis of the nomadic cells. Thus, when a culture of a bacteria is injected into the peritoneum of the lower animals, a very definite sequence of events takes place. The peritoneal fluid normally contains some small mononuclear cells, probably of endothelial origin, and a few polynuclears. For an hour or so after the injection these cells are diminished in numbers, and the eosinophiles disappear altogether. The exact cause of this diminution is not quite clear. It may be due to the dilution of the peritoneal fluid by the solution injected, or to the destruction of the cells, or to their clumping together on the omentum, but in any case is not due to negative chemotaxis. For the next two hours or so there is a gradual increase of the polynuclears, at the end of that time an influx of small mononuclears, until in about six hours these and the polynuclears are present in approximately equal numbers. After this the mononuclears (which are probably budded off from the peritoneal cells, and are thus endothelial in origin) gradually become larger, forming what Metchnikoff calls macrophages; then the fluid slowly becomes concentrated, and the polynuclears gradually disappear, many being ingested by the large mononuclears. Finally these too disappear, but it takes about a fortnight for the animal to revert to its normal condition. Both varieties of cells—endothelial and polynuclear—take part in ingesting the cocci.

Briscoe has shown that a remarkable series of processes also occurs when organisms, etc., are injected into the alveoli of the lungs. It varies according to the substance injected, and we may take the result of the injection of the potato bacillus as an example. In the first hour and a half phagocytosis is very active, the bacilli being taken up exclusively by the pre-existing alveolar epithelial cells. Up to this time practically no polynuclears have made their appearance, but they now commence to be attracted into the alveoli, where they occur in large numbers for about twenty-four hours. They take, however, but a small share in the phagocytosis of the bacteria, and are themselves taken up by the alveolar cells. Some proliferation of these latter cells occurs, and the eosinophiles increase for the first twenty-four hours, and then gradually diminish. We cannot trace the reason for this



sequence of phenomena, but it is evident that the division of labour is carried to a high pitch amongst the phagocytes, and that there must be some controlling influence which regulates the appearance of the cell when it is required.

We must now turn to a discussion of the importance of these facts in connection with immunity as it appeared to the pathologists before the discovery of the antibodies. Much of this is

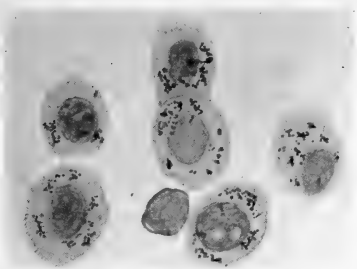


FIG. 50.

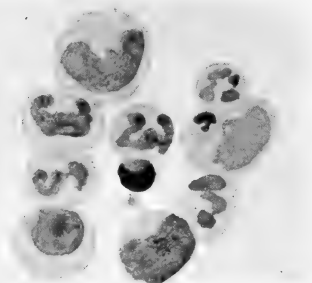


FIG. 51.

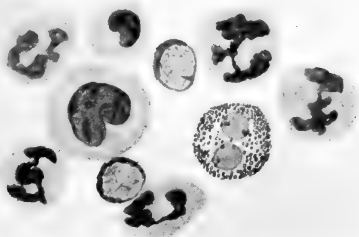


FIG. 52.

FIGS. 50 TO 52.—FROM SCRAPINGS FROM THE LUNGS HALF AN HOUR, TWO HOURS, AND TWENTY-FOUR HOURS AFTER THE INJECTION OF POTATO BACILLI INTO A BRONCHUS. (From films lent by Dr. Briscoe.)

The bacilli, which occurred in large numbers in the alveolar cells half an hour after injection, are not shown.

mainly of historic importance, but it is of extreme interest, and it is to the controversy which occurred between the cellular and cellulo-humoral schools that we owe much of our knowledge of the processes of inflammation and of the functions of the leucocytes. This controversy was carried out with great skill on both sides, and was the means of suggesting numerous experiments of much beauty and ingenuity. To begin with, Metchnikoff's

position was simple and logical. He pointed out that in mild and non-fatal infections phagocytosis usually occurred, and the bacteria could be readily seen inside the leucocytes, whereas in fatal ones little phagocytosis took place, if any. He therefore enunciated the paramount importance of the process in immunity, and at one time considered it would cover the whole field of the phenomena.

But his conclusions did not pass unchallenged, and the supporters of the humoral school adduced numerous examples of recovery from infection where little phagocytosis could be observed, and went farther, and showed that recovery might occur under conditions in which phagocytosis was impossible. The best experiments of this sort were those of Baumgarten, which were repeated by Sanarelli. These observers placed non-virulent bacteria in the peritoneal cavities of animals enclosed in bags of collodion or other substances which would permit the free diffusion of the peritoneal fluids, but would prevent the access of the leucocytes, and they found under such conditions that the bacteria were completely destroyed. This was, of course, an example of bacteriolysis of a type with which we are now familiar. Other observers, including Metchnikoff himself, failed to get these results; but in an experiment of this sort a positive result is of more value than a negative one. It is possible, for example, that the walls of the bags which Metchnikoff prepared may have been sufficiently impermeable to prevent the access of the bacteriolytic substances. Then other observers found that bacteria often underwent changes indicative of death and destruction *before* they were taken up by the phagocytes. Thus Nuttall found that when attenuated anthrax bacilli were placed in a fine tube in the tissues of a rabbit's ear, the organisms showed degeneration forms before they were taken up by the leucocytes, and thought that they were injured by the serum before being ingested. We have already alluded to this experiment as one of the starting-points of the researches on the alexins. As a result of experiments such as this, the humoralists relegated phagocytosis to a part of quite secondary importance. They held that the injury or death of the bacteria by the humours of the body was the important factor, and admitted only that the phagocytes acted as scavengers to remove the dead or disabled organisms. To this Metchnikoff responded by allowing a leucocyte to take up a living and virulent anthrax spore, and then isolating the leucocyte

and planting it on a suitable culture medium, on which the cell died; but the spore survived, showing that it was taken up without any previous injury. He also traced in a very clear and full manner the steps by which a tubercle bacillus of absolutely normal appearance, and apparently vigorous and healthy, undergoes

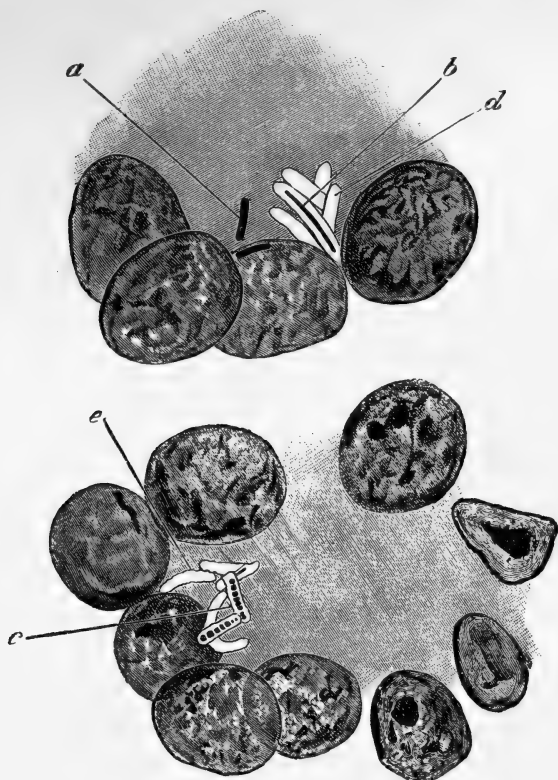


FIG. 53.—PROCESS OF ABSORPTION OF TUBERCLE BACILLI IN GIANT CELLS.  
*a*, Unaltered bacilli; *b*, *c*, *d*, and *e*, various stages in the process.

degeneration, death, and absorption in the giant cell. His case was proved to the hilt in the case of certain bacteria, whereas his opponents proved theirs in others. They were dealing with immunity of different types, and the time was not ripe for the solution of the problem.

The views of another school which sprang up at this point, and which attempted to reconcile these two views, are of more impor-

tance, in that they approach more closely to the modern theory of opsonic immunity, and, indeed, are as close an approximation to it as could have been formed in the then state of knowledge. They were as follows: The importance of phagocytosis was recognized, and it was also admitted that bacteria were frequently prepared for ingestion by dissolved substances, but it was thought that these substances emanated from the leucocytes. The phagocytes were thought to produce an alexin which injured the bacteria, and then to devour them. Baumgarten's collodion-bag experiments were explained by supposing that the leucocytes which collected round the bags in the peritoneum gave off alexin, which diffused through and was sufficient to kill the leucocytes, though more slowly and with more difficulty than if the phagocytes had been able to give the *coup de grâce*. In dealing with organisms of very low virulence it was admitted that phagocytosis might be all-sufficient.

Some of the experiments pointing in this direction may be briefly referred to, though many have been alluded to before in the chapter on the complements. Nuttall continued his experiments on the destruction of anthrax bacilli by a comparison of the action of blood and serum, and found that the latter was enormously the more powerful; and this he explained by the assumption that the protective substances are given off in the solution of the leucocytes which occurs in the process of clotting, and many other experiments were forthcoming in support of this view. But the most beautiful researches were those of Kanthack and Hardy, alluded to previously, but now to be described at greater length. When anthrax bacilli are placed in frog's lymph and examined microscopically, the first phenomenon which occurs is the approach of the eosinophile leucocytes to the bacilli. These cells lose their granules, and at the same time the bacilli begin to show signs of degeneration, the inference being that the granules are dissolved, and that the solution acts injuriously on the bacteria—*i.e.*, is alexin. The next step is for the hyaline cells to approach the area of conflict, and to fuse with the eosinophiles to form a plasmodium around the bacilli. Then the oxyphile cells separate themselves from the plasmodium and move away, and then the hyaline cells can be seen to have taken up the bacilli, fragments of which can still be seen within them. Lastly, a number of cells with basophile granulations are attracted, but their function is unknown. It is obvious that there is here a

division of labour, the hyaline cells being the phagocytes and the eosinophiles the mother cells of the defensive substances. The granules may be regarded as a "pro-enzyme" stage of alexin.

It must not be thought from this experiment that it was held that the eosinophile cells are always the cells which secrete the

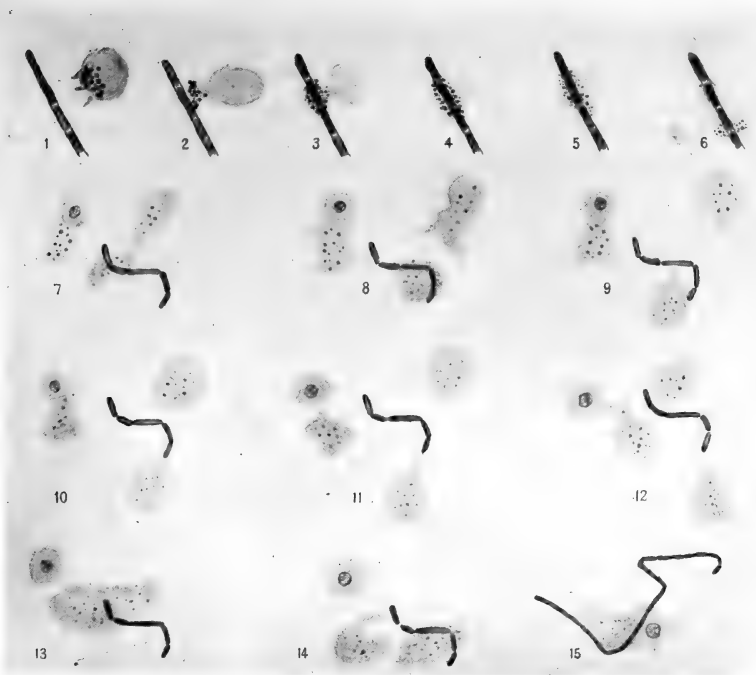


FIG. 54.—KANTHACK AND HARDY'S EXPERIMENT. (Original.)

1-6, An oxyphile leucocyte attacking a thread of anthrax bacilli; the figures were drawn at intervals of one and a half to two minutes, the whole sequence occupying twelve minutes. 7-14, A thread repeatedly attacked by three oxyphile leucocytes, one of which formed a plasmodium with a hyaline cell when observations were commenced; drawn at intervals during a period of one hour. 15, A thread attacked by a plasmodium, consisting of an oxyphile and a hyaline cell, the former having lost its granules. The alteration in the bacilli, which was quite clear in the specimens, is not shown.

The whole drawn from one preparation, the first series immediately after it was put up, the second after half an hour, and number 15 after two hours.

alexin. This is certainly not the case in man, where these cells play a very small part in inflammatory reactions of ordinary type. Here we must assume that, if a similar process occurs at all, the

injurious substance is provided by the polynuclears, which thus play both parts.

Kanthack's experiment is the best and most direct evidence of the extracellular injury of bacteria by substances derived from the leucocytes occurring as a preparation for phagocytosis.

Metchnikoff resisted these views for a time, but soon had to admit that phagocytosis is not the only factor in immunity; and he then altered his theory in an ingenious way, and regarded the extracellular injury or solution of organisms as being essentially the same process as that by which they are digested after being taken in by the phagocytes. He considers that bacteria, red corpuscles, etc., after being taken in, are digested by the action of a proteolytic enzyme which he calls "cytase"—a term which has been already alluded to as a synonym for complement or alexin. Of this there are two sorts: macrocytase, which is formed by the macrophages, and which digests corpuscles, cells, etc.; and microcytase, formed by the polynuclears, and powerful against bacteria. Ordinarily these enzymes are restricted to the cells which form them, and where ingested bodies are contained in vacuoles, these latter contain a solution of the suitable cytase; but when solution of the phagocytes occurs the cytase is set at liberty, and may then exert its action on cells or bacteria which are lying free. Metchnikoff regards this as a process of much less importance than phagocytosis, and points out that the solution which it brings about is rarely complete: thus, when bird's corpuscles are ingested, they are entirely absorbed, nuclei and all; whereas when they are acted on by a hæmolysin (which Metchnikoff regards as a macrocytase), the nuclei remain. This is certainly true as regards the action of most sera on bacteria, solution being rarely complete, and it is only in the highly potent sera obtained by prolonged immunization to certain bacteria that complete disappearance of the bacteria occurs as a result of the action of serum; yet when taken up by the leucocytes they are digested altogether, sometimes with great rapidity.

The difference between these views and those of the cellulohumoralists is roughly this: Metchnikoff looks upon the protective substance as a digestive enzyme which has for its object the transformation of the foreign cells, etc., into proteids suitable for the nourishment of the phagocyte; whereas most bacteriologists regard them as being allied to the toxins rather than to the enzymes, and as being specially intended for the defence of the

body against invaders. The point is one of theoretical interest rather than of practical importance, and we have already pointed out that the complement is apparently used up in its activity, and not set free to attack other molecules, as is the case with the enzymes.

Another minor point is that Metchnikoff seems to regard the setting free of cytase as only occurring when the mother cell is dissolved, whereas most of the bacteriologists who admit the origin of alexin from leucocytes regard it as a product of its secretory activity. The point has been referred to before. Metchnikoff explains the phenomena which occur in immunized

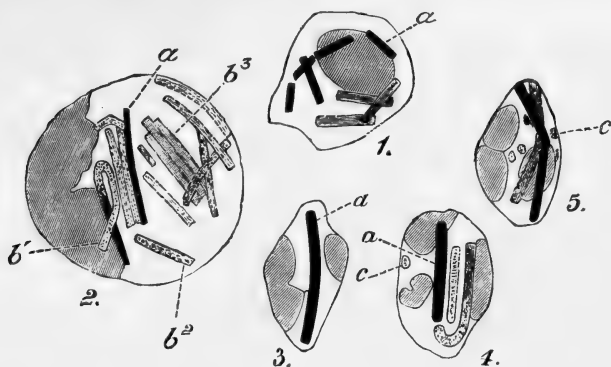


FIG. 55.—PROCESS OF ABSORPTION OF ANTHRAX BACILLI IN THE LEUCOCYTES OF THE PIGEON. (Metchnikoff.)

(Showing various stages of alteration of the bacillus whilst in the protoplasm of the leucocytes.)

as opposed to normal animals in this way: We will take the absorption of bird's corpuscles from the peritoneum as an example. When the injection takes place into normal animals, there is no extracellular destruction of the corpuscles (hæmolysis), because there is no cytase free in the peritoneal fluid, no corpuscles having been broken down; the corpuscles are taken up by the phagocytes, but with some difficulty, since they have not been prepared in any way for the process. When a second or third injection is given, some hæmolysis occurs, and this is because the cells of the peritoneum are broken down by the brusque introduction of the corpuscles; this breaking down is termed phagolysis, and is regarded as being a necessary preliminary to the liberation of the cytase. The fresh leucocytes which arrive now proceed to ingest the corpuscles with great

avidity, since they are already partially digested. We should explain the phenomena very differently: the hæmolysis is a result of the action of amboceptor and complement, and the phagocytosis of the action of an opsonin.

The explanation of bacteriolysis and hæmolysis by means of complement and amboceptor might appear to be difficult on the theory of the reference of the whole process to the digestive action of the phagocytes, but Metchnikoff has applied the researches of Pawlow in a very ingenious way to show a parallelism between cytolysis and digestion. It will be remembered that pancreatic digestion depends upon the action of two substances—an enzyme, protease, which occurs in the pancreatic juice, and another substance, enterokinase, which occurs in the succus entericus. Metchnikoff regards the protease as analogous with cytase, and the enterokinase as analogous with complement or *substance sensibilatrice*. Delezenne showed (though I believe his results are not universally accepted by physiologists) that protease has no power of attaching itself to proteids, whereas enterokinase has such a power, and the substance thus sensitized can then be attacked by protease. This, if true, is exactly similar to the action of amboceptor and complement. We may suppose, then, that amboceptor represents some substance used by the phagocyte to assist the action of cytase or alexin on the bacteria, etc., and normally retained in the protoplasm.

When an organism which is easy to deal with is injected it is taken up by the phagocytes and dealt with in their protoplasm, no preparatory action being necessary. Under other circumstances, when the infection is a more virulent one, some of the phagocytes are killed and dissolved, and their digestive enzymes escape, and partially digest the bacteria, which are then ready for phagocytosis. When there is a balanced contest of long duration another substance is formed, which, under normal circumstances, is not necessary for intracellular digestion, but which facilitates it in difficult cases; this also may escape into the juices, and still further facilitate the preparatory stages of digestion. Lastly, as a rarity, enough of these soluble substances may be set free to dissolve the bacteria altogether, and render phagocytosis unnecessary. To Metchnikoff cellular digestion and nutrition are the important factors in immunity; extracellular action is a less important and occasional phenomenon, and occurs mainly or entirely as a preparation for phagocytosis. His theory



is logical, complete, and well supported by evidence, but it does not take into account the more recent work of Sir Almroth Wright and his followers, and this now calls for discussion before the rôle of phagocytosis in immunity can be profitably discussed further.

It may be admitted that Wright did not discover the fact that serum may aid phagocytosis by acting on the bacteria; this had been already shown by Denys and Leclef in 1895, by Mennes and by Markl. And Neufeld and Rimpau had carefully investigated the same property in the serum of animals immunized to streptococci and pneumococci, and had described their bacteriotropic substances, which are apparently identical with what we now know as thermostable opsonins. This does not detract in the least from the credit due to Wright, who by devising a simple quantitative method of examination, readily applicable in clinical medicine, made a very great advance in our knowledge of the theory of the subject, and has added a most important and useful method of examination of the blood. The credit for the introduction of the use of vaccines in the treatment of established disease (as opposed to its prevention) is, of course, due to him alone.

The name *opsonin* (*opsono* = I cater for, I prepare for food) is given to substances which occur in the serum and have the power of preparing bacteria and other cells for ingestion by the leucocytes, and which are, or are held to be—for there is no absolute proof—different from the substances which we have previously considered. We shall discuss this question of identity or non-identity subsequently, and shall be content at present with saying that, whereas bacteria that have been exposed to the action of alexin are, or may be, obviously injured, a bacterium may be saturated with opsonin without being injured in the least, and may still retain its viability and virulence uninjured.

The fundamental experiments of Wright and Douglas were of this nature, and they are easy to repeat and unimpegnable in accuracy. An emulsion of leucocytes, free from serum, is prepared by receiving blood in normal saline solution containing citrate of soda, centrifugalizing, removing the supernatant fluid and replacing it with saline solution, mixing and recentrifugalizing. This process must be repeated until all trace of serum is removed, and the top layer of the deposit is then pipetted off, and will be found to be rich in leucocytes.

The first experiment is to determine whether leucocytes thus free from serum are able to ingest bacteria. To this end they are mixed with an emulsion of staphylococci or tubercle bacilli, enclosed in a capillary tube and incubated at 37° C. for a quarter of an hour. At the end of that time the emulsion is expelled, and films are prepared and stained in the ordinary way. It will be found that the leucocytes have taken up very few bacteria, if any. It is obvious, therefore, that phagocytosis goes on to a very small extent in the absence of serum. Some species of non-pathogenic



FIG. 56.—ON THE LEFT, A PORTION OF AN OPSONIN FILM (OF PNEUMOCOCCI); ON THE RIGHT, A PORTION OF A SIMILAR FILM, TAKEN FROM A PREPARATION IN WHICH NO SERUM WAS USED. (Original.)

bacteria are taken up well in the absence of serum, and one micrococcus which I have met with was not ingested under any circumstances whatever.

Secondly, a mixture similar to the above is prepared, but with the addition of one volume of serum, so that the mixture consists of an equal volume each of leucocytic emulsion, bacterial emulsion, and serum. This is incubated and examined as above, and it will be found that many bacteria are taken up; the number depends on the thickness of the emulsion and on the source of the serum, but if the former be rich and the latter potent there may be an average of twenty or even far more per polynuclear. The bacteria which are not ingested show no signs of digestion, there being no loss of sharpness of contour or of staining activity.

It is clear, therefore, that serum has a great power in aiding phagocytosis. Is this due to an action on the bacteria or to a stimulation of the leucocytes? Two experiments show that the former occurs; there is but little direct evidence for or against the latter.

In one experiment Wright (having shown that the power of the serum is destroyed by heating it to  $55^{\circ}$  C. for thirty to sixty minutes, or to  $60^{\circ}$  to  $65^{\circ}$  C. for fifteen minutes) allowed serum to act on bacteria, and then heated the mixture until the activity of the serum was removed. He found bacteria thus treated were taken up readily. This must have been due to an action which the serum had exerted on them *before* it was heated, and any action on the leucocytes is out of question, since they were only acted on by heated and inactive serum.

Another and even better proof of the same fact may be obtained by acting on bacteria with serum, centrifugalizing and removing all trace of the latter by repeated washings with saline solution. Bacteria thus treated are taken up with great readiness, and here no free serum at all comes into contact with the leucocytes.<sup>1</sup> Opsonin, therefore, combines with bacteria, and Bulloch showed that this process goes on at ordinary temperatures and at  $0^{\circ}$  C.<sup>2</sup> Bacteria which have once been acted on by opsonin ("opsonized") may be heated to  $60^{\circ}$  C. for five hours, and are still assimilable by leucocytes; this shows that they are profoundly affected, but they may be absolutely unchanged in appearance.

There is no method by which an absolute measurement of the amount of opsonin present in a specimen of blood can be made, but comparative measurements can be made easily enough by the process elaborated by Sir Almroth Wright. In order to do this it is necessary to have as a standard either the serum of a normal person, or preferably a mixture of sera from several normal persons, so that slight individual variations or abnormalities may be ruled out. The emulsion of leucocytes ("cream") is prepared as described above, and the emulsion of bacteria made by stirring a little of a young culture of the organism in question in some saline solution, taking care to remove clumps by sedimentation or centrifugalization. When tubercle bacilli are being used it is most convenient to employ dead and dried bacilli, which are

<sup>1</sup> This experiment was performed by Markl in 1903, using plague bacilli.

<sup>2</sup> This is not altogether confirmed by Ledingham's more recent work, which is discussed subsequently.

ground up in a mortar with saline solution before use. The mixtures of leucocytes, bacteria, and serum are made in capillary pipettes mounted with an indiarubber nipple, and furnished with a unit mark about 1 inch from the free tip, which is drawn to a fine point. The process is as follows: As much cream as will reach to the unit mark is drawn into the pipette, then a little air (to serve as an index), then a unit of the emulsion, another bubble of air, and finally a unit of serum. These are then blown out on to a glass surface, mixed intimately together, sucked into the tube, the end of which is now sealed. The tube is now placed in the incubator and the time accurately noted. Then the process



FIG. 57.—WRIGHT'S CAPILLARY PIPETTES, AS USED IN DETERMINATIONS OF THE OPSONIC INDEX. (Emery's "Clinical Bacteriology and Hæmatology.")

The small figure shows the tip magnified. The middle figure shows the pipette charged with leucocytic cream (in this case two volumes are shown), emulsion of bacteria, and serum. In the lowermost figure these are mixed together and the tip sealed.

is repeated in exactly the same way, but the control serum is used instead of that which is being investigated. Each pipette is incubated for exactly the same length of time, removed from the incubator, the tip broken off, the contents expelled, and films made. These are obtained in a suitable way, examined under the microscope, and the number of bacteria which have been taken up by 50 or 100 polynuclear leucocytes is counted in each. Thus we may find that in the control specimen (in which healthy blood was used) there are 300 bacteria in 100 leucocytes; in this case we say the "phagocytic index" is 3. In the other specimen (in which the patient's blood was used) we might find 150 bacteria in 100 leucocytes, giving a phagocytic index of 1.5.

We see in this case that the patient's blood has but half the opsonic power of normal blood; this we express by saying that the *opsonic index* is 0.5. The opsonic index is obtained by dividing the number of bacteria found in a certain number of leucocytes in the films made with the patient's serum by the number of bacteria in the same number of leucocytes in the films with the control serum, and expresses the phagocytic power of the patient's serum as compared with that of a healthy person. It is not necessarily an exact measure of the amount of opsonin, since on dilution of a serum the opsonic index falls at first slowly and then more quickly, forming a flat-topped curve when plotted out in the usual way (see Fig. 59, p. 265).

Other methods for the estimation of the opsonic index have been suggested, and require some mention. In the earliest method—that of Leishman—the patient's blood was mixed directly with an emulsion of the bacteria in normal saline solution in equal parts, and a drop of the admixture placed on a slide, covered with a cover-glass, and incubated for a definite time. A control specimen was prepared in a similar way, using normal blood. After the incubation, films were prepared by sliding the cover-glass off the slide, stained, and a count made as in the method now in use. A similar but rather better method is also employed, and is extremely convenient in some cases. The bacterial emulsion is prepared as above, the organisms being suspended in normal saline solution containing sodium citrate. A mixture of this emulsion and of the patient's blood in definite amounts (usually equal parts) is prepared, sucked into the pipette (the tip of which is sealed), and incubated for a quarter of an hour or twenty minutes. The process is the same as Leishman's except that the mixture is incubated in a pipette, and not between slide and cover-glass. A control is also prepared, using the same emulsion and normal blood, and is also incubated for a quarter of an hour. At the end of this period films are prepared, and the process finished in the ordinary way.

This method is theoretically more accurate as a test of the phagocytic activity of the patient's blood as compared with normal blood than is the opsonic index as determined in the ordinary way, in which leucocytes from the same source are used in both determinations—*i.e.*, in that of the patient's serum and in that of the control. Thus, if in any case the leucocytes were so injured that they had very little phagocytic power, the opsonic

index as determined by Wright's method might nevertheless be normal; yet this blood might have but little power of destroying bacteria which gain access thereto. There is some experimental evidence that alterations in the power of the leucocytes do actually occur; thus Shattock and Dudgeon, in some experiments with granules of melanin (which, like bacteria, require to be opsonized before they can be taken up by leucocytes), found that either more or less might be taken up by the patient's leucocytes as compared with normal ones, using the same serum in all cases. The numbers varied between 0.46 and 2.9, taking the normal number as unity. It must be pointed out that this method does not give the opsonic index of the serum, and that in cases, *e.g.*, in which a low result is obtained it affords no information as to whether the leucocytes or the serum is at fault, or both. Further, there is a possible error owing to the possible difference in the number of the leucocytes in the unit volume of the two specimens of blood. Where the patient has a leucocytosis—and this is very common in the type of case in which opsonic estimations are required—the difference may be very great. The result of this has not been fully elucidated, but it is obvious that where the bacterial emulsion is not very thick the number available per leucocyte is very different in the two cases. This is a point worthy of consideration in the determination of the opsonic index by Wright's method.<sup>1</sup> When the bacterial emulsion is very dilute a large error is introduced, and even if very large numbers of leucocytes are counted the results are untrustworthy. The best results theoretically would be obtained where the emulsion was so thick that every leucocyte would take up as many bacteria as it was capable of doing in the given time. This is impracticable, however, as the labour in counting leucocytes containing very many bacteria is great, and the error in counting is also large. Probably the best results are obtained where the phagocytic index in the control is about 4, and it is a good plan to perform an orientating experiment to determine the appropriate strength of the emulsion before commencing a large series of opsonic determinations.

<sup>1</sup> It has been investigated by Ruth Tunnicliffe, who finds no very great differences in a series of estimations in which the bacteria (*diphtheria bacilli*) varied from 125,000 to 1,000,000 per cubic millimetre, all the other factors being constant; and by Walker, who finds that the index rises greatly if a thicker emulsion is employed.

Another modification of Wright's method, introduced by Simon, concerns the method of counting only. A large number of leucocytes are counted, and are classified simply into those that contain bacteria and those that are free. Of course, the emulsion must not be too thick, or practically all the leucocytes will have taken some up. The process is repeated with the control, and the results compared; thus, if in the control film 25 per cent. of leucocytes were empty, and in the patient's film 50 per cent., the index would be  $\frac{25}{50} = 0.5$ . A comparison of the results obtained by this method and by careful counting show that they are fairly comparable, and the process may be used where it is only necessary to determine whether the index is high or low.

Another and more important method is that of dilution or extinction, as introduced by Dean and by Klien. It is especially useful in the case of bacteria, such as *B. typhosus* and *V. cholerae*, which are dissolved by fresh serum when but slightly diluted. Further, when an attempt is made to determine the opsonic index to the former, and the pipette is incubated for but five minutes, numerous shadows and partially digested bacilli are seen within the leucocytes, thus introducing a new and very important error. In order to avoid this, Klien determines the degree of dilution of the serum necessary for the complete extinction of its opsonic action. In preparations in which no serum is used the phagocytic index is usually below 0.5, and the serum to be tested is diluted until the degree of dilution is found, which gives a phagocytic index no higher than this. Working by this method, Klien obtained results very different from those obtained by Wright's method. In the process of immunization of a rabbit the index (by the latter method) remained low, varying only between 0.82 and 1.65, whereas by the process of dilution it was seen to be actually greatly raised. Before the commencement of the immunization the opsonic power of the serum was extinguished when the latter was diluted thirty times, whereas afterwards it did not disappear until diluted 3,072 times. It appears clear that in the case of bacteria like this the results obtained by Wright's method are quite misleading. Klien states that the bacterial emulsion should be a thick one, and should be of about the same density in successive experiments, if these are to be comparable. The main objection to this method is its tediousness: many pipettes have to be prepared, and many films examined.

It has an advantage over Wright's method even in cases in which the bacteria are not dissolved in the serum or leucocytes, in that it provides a definite measure of the amount of opsonin present, which the ordinary method does not do, as is shown by the fact that the opsonic index of a mixture of equal parts of serum and normal saline solution is more than half that of undiluted serum.

An enormous number of opsonic determinations have been carried out, and the results have been of extreme interest. It is

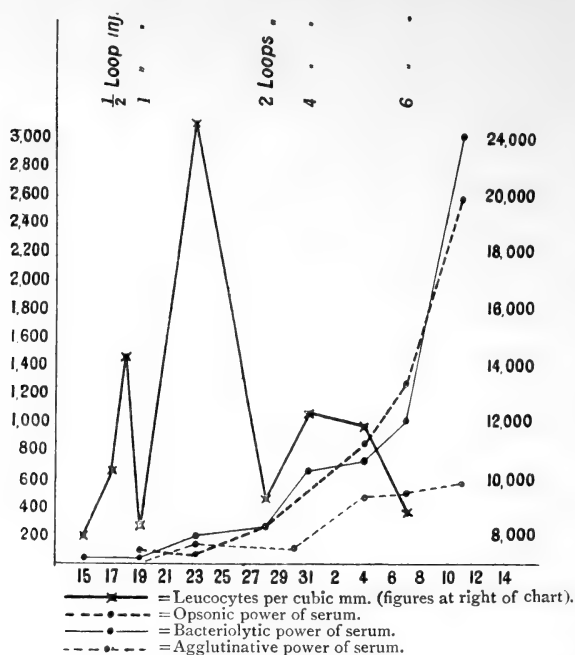


FIG. 58.—INFLUENCE OF INOCULATION OF TYPHOID VACCINE ON THE OPSONIC POWER OF THE SERUM OF A RABBIT, AS SHOWN BY THE DILUTION METHOD. (After Klien.)

found that the indices of healthy persons is approximately the same, and does not vary much from day to day. In the case of tubercle a very large number of determinations of the indices of normal persons have been made, and it is found that, with one or two exceptions, due perhaps to accidental errors in technique, they all lie between 0.8 and 1.2, taking 1 as a standard. In reality they agree very much more closely than this, for the great



majority lie much nearer to 1. When the estimations are carefully carried out, very few will be found below 0.95 or above 1.05. We may regard the opsonic index for a given organism as a definite quantity in a healthy person. Some sera are lower or higher than others, but the difference is but slight, and the index of the same person is found to show but slight daily variations as long as he remains in good health. A few observations go to show that the index is slightly lowered in persons who, without being ill, are in a

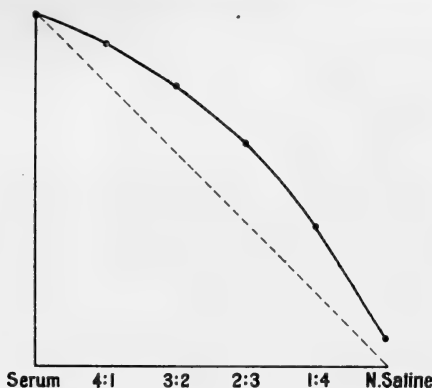


FIG. 59.—SHOWING EFFECT OF DILUTION OF NORMAL SERUM ON THE NUMBER OF BACTERIA TAKEN UP. (Original.)

state of lowered vitality, and that the onset of a mild disease, such as a cold, may cause a fall in the index to tubercle or other disease.

When the patient suffers from a disease due to a given organism, and his index is tested against this organism, the results obtained are of extreme interest. Taking the acute diseases first, we find that as a rule the index is low at the commencement of the illness, and that it rises, either gradually or suddenly, when recovery takes place; and in some cases there is a definite correlation between the course of the index and that of the disease. For example, Macdonald has shown that in an attack of pneumonia the opsonic index of the patient's serum to the pneumococcus remains at a constant low level until the crisis is reached, when it shows a sudden rise, attaining a point above the normal level. It remains elevated for a short time, and then relapses to normal or below normal. It is difficult to believe that the rise in the opsonic index and the consequent increase in phagocytosis which w

should expect to be caused thereby is not the cause of the crisis and the patient's recovery. The short duration of the high level of the index is interesting, as we know that the immunity left after an attack of pneumonia is but temporary.

A gradual rise of the index often takes place in staphylococcal diseases—*e.g.*, boils; and when the index is traced from day to day, it may be seen that it is low to begin with, during the onset and increase of lesion, but that it rises more or less gradually until it

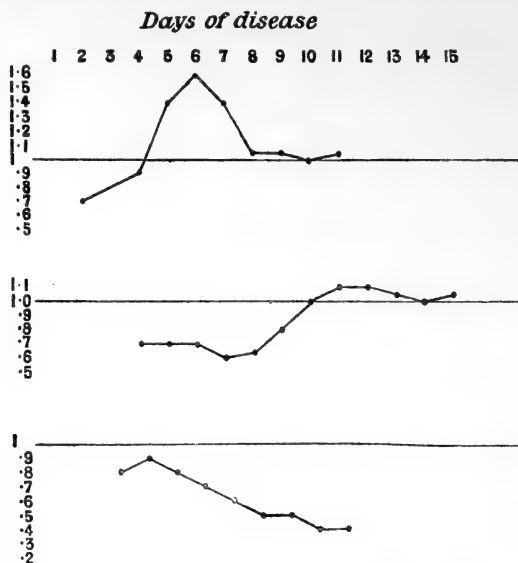


FIG. 60.—TYPES OF REACTION OF THE OPSONIC INDEX IN PNEUMOCOCCIC INFECTION. (After Eyre.)

(a) Immediate, as seen in mild diseases; (b) delayed; and (c) progressive decline, as seen in severe and fatal infections.

reaches a point well above normal. At the same time the disease begins to improve, suggesting again that the phagocytosis dependent on the amount of opsonin in the blood is the actual cause of the recovery. Very many observations of this type have now been made with many organisms, and as a general rule we may say that in acute diseases (*excluding tubercle*) the index is, as a rule, low during the onset and culmination of the disease, and raised during involution and recovery. Exceptions may be met with, but the sequence of events happens too often to be a mere coincidence (see Figs. 60, 61, 63, and 64).

Hence the opsonic school of immunity has formed a theory which may be enunciated as follows: The immunity to certain organisms (not to all) depends on phagocytosis, and this can only take place in virtue of the preparation of the organism by the action of opsonin. Where this substance is present in normal amount the person is sufficiently immune to resist ordinary infections; but if for any reason the amount is lowered or the

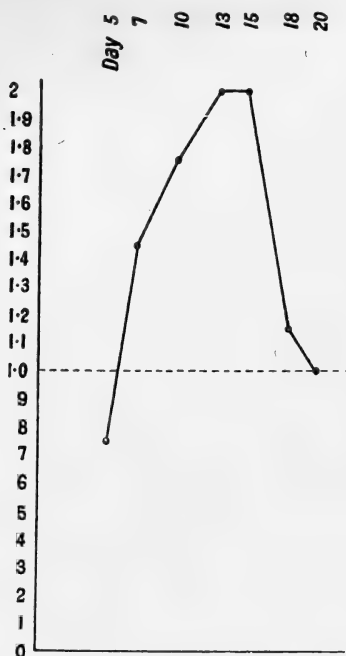


FIG. 61.—OPSONIC INDEX IN DIPHTHERIA. (Tunncliffe.)

infection very virulent, phagocytosis cannot occur, and the disease progresses. There is then a new formation of opsonin, just as there is of other antibodies, and this goes on until there is sufficient to sensitize all the bacteria and render them amenable to phagocytosis, when recovery occurs. When this does not take place the patient's phagocytes cannot ingest the bacteria, and the disease progresses.

There is one assumption that will require critical consideration subsequently, and that is that opsonin is an antibody.

The behaviour of the index in chronic infections is different,

and is difficult to explain on the opsonic theory of immunity. In a chronic staphylococcic lesion, such as acne, the index may be low, normal, or high, and this is also the case to a most marked extent in tuberculosis. Wright classified the cases of this disease into two groups: (1) strictly localized tubercle, such as lupus, mild glandular cases, tuberculous abscesses, etc.; (2) cases associated with constitutional disturbances. In the former he found the index uniformly low (from 0.13 to 0.88), whereas in the latter there was great variation, the index being below normal, or as high as 2 or more. Further researches, however, have not confirmed this, and the indices of patients with lupus will often be found very high. As a rule, however, the patients with localized tubercle, if kept at rest in bed, will be found to have a constant index, whereas in those with a progressive disease it will be found to vary from day to day, being often very high.

These variations are attributed to auto-inoculation—*i.e.*, to the discharge from the lesion of a few bacteria or of a small dose of bacterial toxin, which makes its way into a region suitable for the elaboration of a further amount of opsonin, acting just as an injection of a vaccine, and causing a negative, followed by a positive phase. When the patient is kept absolutely at rest in bed this does not occur, or only to a comparatively slight extent, and the index is more or less steady. If, however, the patient be allowed to exert himself, even slightly, or if the lesions are gently massaged, specific substances are set free, auto-inoculation occurs, and the index exhibits its characteristic oscillations. It is also dependent to some extent on the temperature, as has been shown by Inman and others, tending (in phthisis) to fall with a rise of temperature, and *vice versa*. In general, a fluctuating temperature accompanies a subnormal index, a rise occurring when the oscillations become less. The injection of a bacterial vaccine may cause a rise of temperature, especially if the amount is large, but does not always, and should not, do so.

In chronic infections a high opsonic index does not necessarily imply that a patient is doing well. In general tuberculosis the index is often normal or elevated, and a rise may occur just before death. This is also the case in acute infections, such as erysipelas, in which a sudden and great elevation may immediately precede the fatal issue (Fig. 63).

These results are difficult to harmonize with the opsonic theory, but Wright points out that it is not sufficient for there to be

enough opsonin in the blood; it must reach the diseased tissue.

Some observations go to show that it may be unable to do this under certain conditions. Thus Bulloch found the liquor puris from a staphylococcic abscess entirely devoid of opsonin to staphylococci. This might have been due to absorption by the bacteria in the pus, so he cleansed the abscess, and, taking the first few drops which collected, found them also very deficient in opsonin. It appears, therefore, that this substance, though present in the blood, was unable to make its way through the

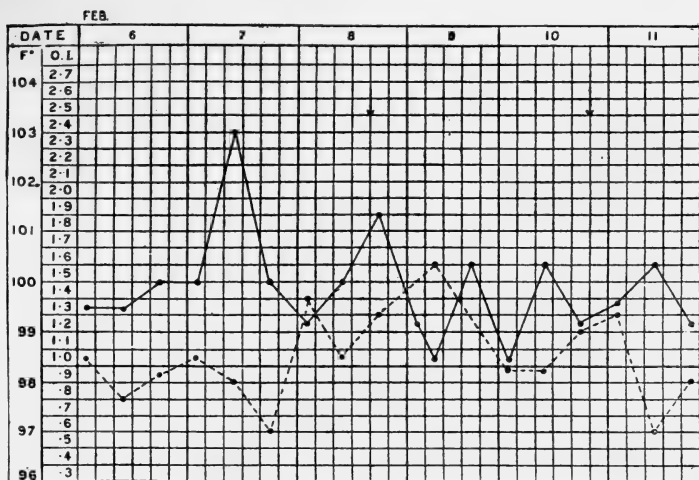


FIG. 62.—SHOWING INVERSE RELATIONSHIP BETWEEN TEMPERATURE AND OPSONIC INDEX IN PHTHISIS. (Inman.)

The continuous line shows the temperature.

wall of the abscess to the place where it was wanted. Again, Wright has shown that the serous fluid in cases of tuberculous pleurisy and peritonitis is very low in opsonin as compared with the circulating blood, and has made use of this fact as a means of diagnosis. It must be obvious that in the case of an extra-vascular object like a tubercle, and especially a caseous mass, that a slight alteration in the opsonic index of the blood can have but a slight immediate effect; any beneficial effect of a high index must be slow in manifesting itself. To remedy this, Wright attempts to flush the morbid tissues with blood or lymph

by diminishing the viscosity of the blood by the exhibition of citrates and other anticoagulants, by the use of hot applications, and by Bier's method of congestion.

The first point which arises in a discussion of the opsonic theory deals with the specificity of the opsonins themselves. Are we to imagine that there is a specific opsonin to each organism, and that during the process of immunization this increases, whilst the others remain constant? Unless this is the case, the theory fails, for we know that immunity *is* specific.

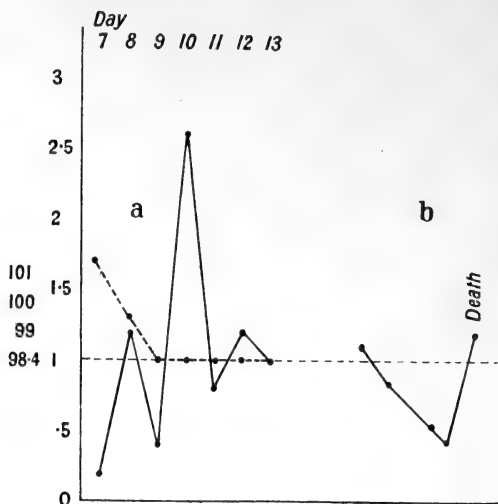


FIG. 63.—BEHAVIOUR OF THE OPSONIC INDEX IN A MILD (a) AND SEVERE (b) CASE OF ERYSIPELAS. (After Tunncliffe.)

The latter shows the preagonal rise; the broken line in the first chart indicates the temperature.

The question may be investigated in two ways—by absorption of the opsonins and by comparison of different sera.

The first method was employed by Bulloch and Western, who added an emulsion of tubercle bacilli to normal serum, and found but a slight reduction of the opsonic index to staphylococci, suggesting the difference of the two opsonins. But these results have not been confirmed by later writers, and it is quite certain that a sufficient amount of tubercle bacilli will remove practically the whole of the opsonin to staphylococci. These experiments tend, therefore, to show that the opsonins are not

specific, and that any immunity due to them would be a general one.

The second method is by a comparison of various sera in their action on various organisms. For example, we may take two sera, and compare them in their action on tubercle bacilli and on staphylococci. If we find uniformly that a serum which is low to one is also low to the other, it will tell strongly against the theory of specificity. This, however, is what we do not find, and it is quite usual to discover that a serum which is very low to the tubercle bacillus as compared with a normal control has a normal index to staphylococci as compared with the same control. Of this there can be no doubt. Further, after the injection of a vaccine composed of the dead bodies of certain organisms, it is usual to find the opsonic index to that organism rise, whereas to others it remains unaltered. This tends very strongly to show that opsonins are specific bodies.

Quite similar results are seen when the behaviour of the opsonic index to two or more bacteria is followed from day to day in a patient suffering from an infection by one of them. Thus, in a patient who was recovering from a severe furuncle the index to staphylococci and tubercle bacilli was observed, with the result shown in Fig. 64.

Here we may regard the tubercle opsonin as being normal throughout, the slight variations met with being well within the range of experimental error. The index to staphylococci, on the other hand, ranged between 0.4 and 1.35, and showed a general parallelism with the amelioration in the patient's condition. It is obvious that the two indices are not due to the presence of a single opsonin.

Reverting to the saturation experiments, we may perhaps explain them as follows: Any opsonin can prepare any bacterium for phagocytosis if it combines with it; but there are different opsonins, with very different degrees of affinity for different bacteria.<sup>1</sup> Thus we may suppose the tubercle opsonin to have a powerful affinity for the tubercle bacillus, a slight one for the staphylococcus, so that the addition of a few tubercle bacilli will

<sup>1</sup> It now seems fairly clear that the explanation of these experiments is that fixation of complement (which in this case acts as an opsonin) takes place. Normal serum contains an amboceptor (=thermostable opsonin) to staphylococci, though in small amount; and this, when combined with staphylococci, will attract all the opsonin to it, the staphylococcus opsonin most powerfully.

remove it from a sample of serum, whereas a large number of staphylococci are required. In this case opsonins will have a sort of modified specificity comparable with that of the agglutinins for the coli group, and this appears to harmonize Bulloch's results with those of later observers.

An example of this selective absorption of opsonins may be given, chiefly to illustrate the methods employed in this class

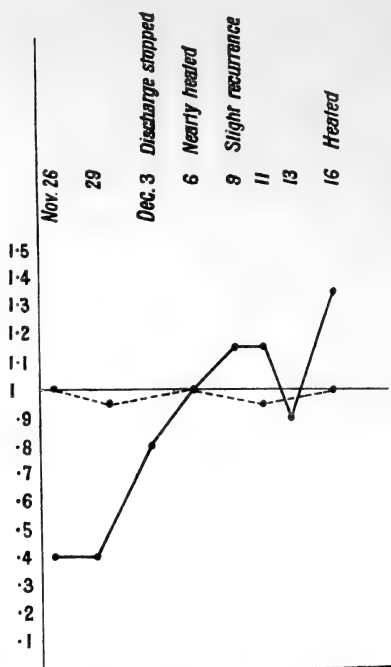


FIG. 64.—BEHAVIOUR OF OPSONIC INDEX TO STAPHYLOCOCCI AND TUBERCLE BACILLI DURING NATURAL RECOVERY FROM AN ATTACK OF FURUNCULOSIS. (Original.)

of experiment. A specimen of normal serum was mixed with an equal amount of very thick emulsion of staphylococci, kept at 37° C. for one hour, and then centrifugalized until all the cocci were removed, leaving the fluid (*a*). A second amount of serum was treated similarly, but the staphylococci emulsion was diluted 100 times (*b*). It was hoped that the staphylococcic opsonin would be completely removed from the first, and only partially removed from the second specimen. This was tested as follows :



Four experiments were carried out, in each of which the fluids (1 unit of each) were mixed with 1 unit of leucocyte "cream," and of a fine emulsion of staphylococci, incubated, and films prepared and counted. Thus:

		<i>Staphylococci in 50 Leucocytes.</i>	<i>Index.<sup>1</sup></i>
1.	Normal serum + normal saline ...	170	1'0
2.	" " + supernatant fluid (b) ..	125	0'69
3.	" " + " " (a) ...	55	0'21
4.	Normal saline + normal saline ...	24	—

These fluids were then tested in exactly the same way with regard to their action on tubercle bacilli. Thus:

		<i>Tubercle Bacilli in 50 Leucocytes.</i>	<i>Index.</i>
1.	Normal serum + normal saline ...	145	1'0
2.	" " + supernatant fluid (b) ...	132	0'93
3.	" " + " " (a) ...	90	0'6
4.	Normal saline + normal saline ...	9	—

Here it is obvious that the staphylococci have removed the staphylococcic opsonins more powerfully than the tubercle opsonin. The strong emulsion removed 80 per cent. of the former and only 40 per cent. of the latter.

A striking example of the fact that there is more than one sort of opsonin is supplied by observations on the hæmopsonins. Most specimens of blood-serum are unable to act as opsonins for the red corpuscles, which are not taken up by the leucocytes under the ordinary conditions of opsonin investigation *in vitro*; but some specimens do possess the power of opsonizing red corpuscles. It is obvious, therefore, that hæmopsonin is not the same as bacteriopsonin.

We must now discuss the nature of these opsonins. Are they familiar substances (*e.g.*, complements or amboceptors) masquerading under a new name, or are they essentially different? And if so, are they antibodies, or are they allied to other protective substances, such as the alexins of the cellulo-humoralists or the cytases of Metchnikoff? This is an extremely difficult subject, and one which has not yet been satisfactorily solved.

The main evidence in favour of the view that they are specific

<sup>1</sup> The indices given are corrected by the deduction of the number of bacteria taken up spontaneously (Expt. 4) from each of the totals. This may be termed the corrected opsonic index, and ought to be given where great accuracy is required.

antibodies is derived from a study of their behaviour when a patient is inoculated with their specific antigens. If, for instance, a patient with a low index for tuberculosis is inoculated with a small dose of new tuberculin (say  $\frac{1}{1000}$  milligramme), consisting of the dead bodies of the tubercle bacilli, a very definite train of phenomena, closely comparable to the results of an injection of diphtheria toxin, is produced. In each case there is an immediate fall

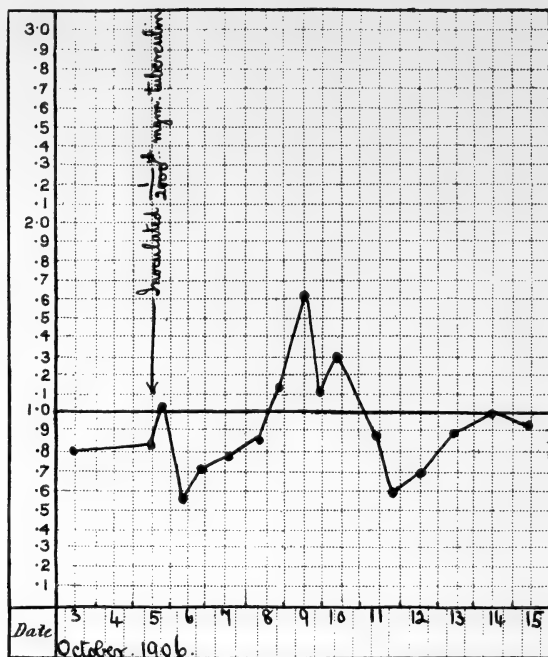


FIG. 65.—EFFECT OF A SINGLE INJECTION OF TUBERCULIN, SHOWING THE "FALSE RISE." (Wright.)

of variable duration, followed by a rise to a higher level than the initial one—in other words, there is a negative followed by a positive phase. (In some cases there is a sharp "false rise" of short duration, which precedes the negative phase, a phenomenon which, as far as I am aware, has not been found with the undoubted antibodies.) Now this rise, as has been already pointed out, is to some extent at least a specific one; an injection of tuberculin does not cause a rise in the opsonic index to staphy-

lococci or pneumococci. There is, therefore, one important feature possessed by the opsonins in common with the antibodies: in each case an injection of the specific antigen causes first a diminution and then an increase in the amount present.

There is, however, an important difference. In the other antibodies—*e.g.*, in diphtheria antitoxin—the amount present in the blood can be raised to a point enormously above that of normal blood by a series of inoculations of suitable doses of toxin at suitable intervals. Here the effect of repeated injections is a cumulative one, the second raising the index above the level which it

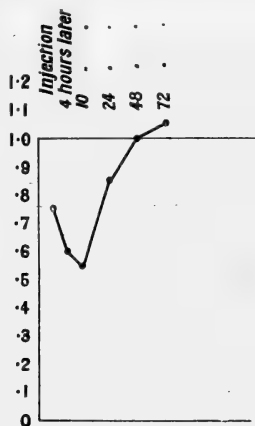


FIG. 66.—RESULT OF A SINGLE DOSE OF STAPHYLOCOCCIC VACCINE, SHOWING NEGATIVE PHASE. (Original.)

reaches after the first, and so on. But in the case of the opsonins to most bacteria there is no such summation of results. An injection of tuberculin may raise the opsonic index from 0.5 to 2 or a little higher, but with a second injection it is not possible to start with 2 as a base and raise the index to 3, and so on. The maximum indices are not very much above the normal level. In the case of tubercle it is very unusual to find an index as high as 2, whilst with the organisms of suppuration, etc., slightly higher figures may occasionally be found.<sup>1</sup> As we have already shown, this does not prove that the amount of tubercle opsonin present

<sup>1</sup> The highest indices of all are met with in the case of the meningococcus. I have seen them exceed 10 in patients treated with vaccine, and higher figures have been recorded. The explanation of these figures will be given subsequently.

in blood never exceeds twice the normal—and the actual amount may be much more—but anything like the enormous amounts which can be obtained when working with antitoxins or agglutinins are never met with in the case of these bacteria at least.

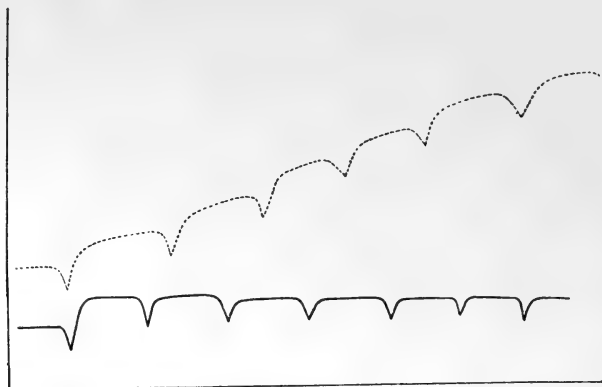


FIG. 67.—SHOWING THE DIFFERENCE BETWEEN THE BEHAVIOUR OF THE TRUE ANTIBODIES (DOTTED LINE) AND OPSONIN TO SUCH ORGANISMS AS TUBERCLE (LOWER LINE) WHEN SUCCESSIVE INJECTIONS ARE GIVEN. (Schematic.)

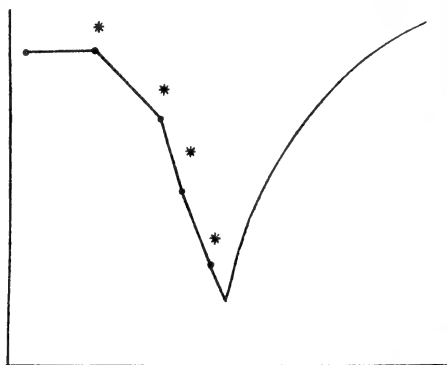


FIG. 68.—SUMMATION OF NEGATIVE PHASES IN OPSONIN FORMATION AS THE RESULT OF INJECTIONS IN RAPID SUCCESSION. (Schematic)

When injections are repeated during the negative phase a phenomenon of summation may be met with, as Wright first pointed out. Here the first injection may lower the index, and the second and third lower it still more, until a very low figure is reached. A phenomenon similar to this may be seen after the injections of toxins (Fig. 68).

It is on these facts that Wright's vaccine therapy, or, as it is sometimes called, opsonin therapy, is based. The object of the treatment is to bring about an immunization of the patient by means of an increase of the opsonin circulating in his blood, and this is achieved by the injection of a suitable vaccine. This consists in all cases of the dead bodies of the bacteria causing the disease. In the case of tubercle Koch's new tuberculin (TR or TE) is used in variable amount, but not usually more than  $\frac{1}{10000}$  milligramme of dry material per dose. In the case of other bacteria the vaccine is prepared by cultivating the organism on a suitable solid culture medium, emulsifying with normal saline solution, and heating to a temperature just sufficient to insure sterility—usually 60° C. for one hour is requisite. The emulsion is then inoculated on to a culture medium, incubated in order to test its sterility, and the number of bacteria which it contains is counted, in order to determine the amount to be used as a dose. Suitable dilutions are then made. The dose varies with different bacteria. Thus, with staphylococci 250,000,000 to 1,000,000,000 cocci may be given, whereas with *B. coli* 25,000,000 is usually enough for the first dose.

The treatment is controlled by a frequent estimation of the opsonic index, and this is supposed to be advisable for three reasons: (1) It avoids the possibility of a summation of the negative phases, and so a worsening of the patient's condition by lowering his immunity to the infective organism. As a rule, the negative phase is but of short duration, but occasionally it is prolonged, and this is especially the case when large doses have been given. I have seen it as long as three weeks in a case of tubercle. (2) It enables a suitable dose to be selected. Thus, if we find a certain number of bacteria cause a long negative phase, the next injection should consist of a smaller one, when the negative phase may be reduced and the rise may be greater. With a very small dose the negative phase may be eliminated altogether, or may be reduced so much that it is overlooked. (3) Whilst the index is raised decidedly above normal it is assumed that the patient is benefiting, and another injection is only required when it begins to fall. As a rough general rule, the injections have to be repeated at intervals varying from a week or fortnight, but individual patients show decided differences in this respect.

Of the practical success of this treatment in certain diseases there can be no doubt, and whatever we may think of its theoretical

aspect, Sir Almroth Wright must receive the greatest credit for its introduction. Before his researches the idea of injecting a vaccine into a patient already suffering from a bacterial disease was unthought of, although, of course, it was well known as a method of producing immunity when disease was feared. The question is often asked, Why inject more staphylococci into a patient who has already too many? The answer may, perhaps, be as follows: The staphylococci which cause the lesion come into contact with dead and diseased tissues only, and it is easily conceivable that these may be very unsuitable to discharge so vital a function as the formation of antibodies, whereas a few cocci injected into the healthy tissues may cause a large amount. This, however, does not explain the benefit which has been observed in some cases of endocarditis and other hæmal infections, for in them the bacteria must be constantly gaining access to the healthy endothelial cells, if to no others. But it is well known that not all the tissues are equally adapted for the production of antibodies; thus, when diphtheria toxin is injected into the blood-stream little, if any, production of antitoxin takes place. As a general rule, when antibodies are required the blood-stream is the worst place in which to inject the antigen, the serous membranes next, and the connective tissues the best. Dr. Whitfield has suggested to me that the reason may be that the stimulation of the opsonins occurs best when *dead* bacteria are injected. Thus in the early stage of the disease only living organisms are present, whilst later we must suppose some are killed or die from some cause, and then the stimulation of opsonin formation begins. The idea is worth considering, but the subject is still obscure.

As regards the nature of these results: In tubercle, speaking from my own experience, I can only report a moderate degree of success, and this only in small lesions, such as tubercle of the iris or cornea and of tuberculous ulcers. I have had but one or two encouraging results and numerous failures with tuberculous glands, bone disease, etc., though others have apparently been more successful. In phthisis there appears to be some slight benefit when combined with other treatment, and tuberculous sinuses sometimes heal very quickly. I should only recommend the treatment myself as an adjunct to other methods, or when surgical interference is impossible or inadvisable.

With the diseases due to acute infections with staphylococci, pneumococci, *B. coli*, and some other organisms, however, the

results are most beneficial. We may often see boils apparently on the point of bursting retrocede in a most striking manner after a single injection of staphylococcic vaccine, and pustular acne is often equally benefited. Localized lesions of pneumococcic origin often clear up quickly under the action of pneumococcic vaccine: thus a case of empyema of the frontal sinus, due to this origin and of four years' duration, was cured in five injections, spread over a period of about two months. Numerous cases of cure of chronic infections of the urinary tract with *B. coli* have been recorded, and some cases of gonorrhœal arthritis have been cured in a remarkable manner. In a case of my own a patient, with five large and numerous small joints affected, was completely cured in three months, after having been crippled for over two years. One or two undoubted cases of ulcerative endocarditis have been cured, and others in which there was a hæmic infection (with streptococci), though the evidence in favour of a valvular infection is less convincing. The results in cases of Malta fever are also very encouraging. As a rule, however, we may say that the special scope of the method is in the treatment of localized infections.

A point of great practical importance, and one that has some theoretical interest as pointing to a high degree of specificity in the opsonins, is the fact that good results are sometimes obtained only when the vaccine used is from a culture of the organism in question from the patient himself. This is sometimes seen in staphylococcic infections; acne is occasionally very resistant to stock vaccines, and yields readily to treatment with an emulsion prepared from a culture from the patient's own pus. This phenomenon is specially marked in the case of streptococci and *B. coli*.

In admitting the success of vaccine therapy, we do not necessarily admit the truth of the theory on which it is based, nor the necessity for the opsonic control of the doses. It is certainly true in general that with acute lesions there is a low opsonic index, and that when amelioration or cure takes place a rise to or above normal occurs, but this is not invariably the case. Thus, occasionally tuberculous patients improve whilst the index remains low, and those with a meningococcal infection often go steadily downhill whilst the index is very high, though in the latter case the symptoms are in general more severe when the index falls. Now it is quite true and perfectly conceivable that the continued existence of a lesion in spite of a very high opsonic

index may be due to a failure of the serum or leucocytes to gain access to the lesions which are densely surrounded by inflammatory material. We have adduced a similar reason to explain the non-success of certain bactericidal sera. But it is otherwise when we find that a patient improves when there is a low index, for here we must admit that even this deficient amount of opsonin is sufficient; and, further, it is impossible to explain the formation of new lesions (*e.g.*, staphylococcic) in patients in whom the opsonic index is high—often very high—on any such grounds. Again, a great rise in the opsonic index not infrequently occurs just before death, as in the chart of the index in a fatal case of erysipelas already figured. The more carefully the opsonic index is considered, the more certain will it appear that a high index is not an indication of immunity; it neither proves that the lesion is undergoing cure nor that a fresh infection will not occur. It may, of course, occur concurrently with other properties in the blood or tissues on which immunity does depend—indeed, since it is commonly due to the presence of a natural or artificial vaccine, it usually does so—but the parallelism between a rise in the amount of opsonins and an increase in the grade of immunity is not absolute. Nor is a low index any proof of lack of immunity, since patients may improve remarkably during a prolonged negative phase. One of the most striking cases of amelioration of a severe case of tubercle which I have ever seen occurred during a negative phase lasting over three weeks. Allen has noted a similar occurrence in gonorrhœal infections, from which, however, he draws the assumption that the clinical signs are a totally unreliable guide to the appropriate time for a fresh injection—a deduction which is logical only if we regard the raising of the opsonic index, and not the cure of the patient, as the object of treatment.

It seems probable, from a consideration of the phenomena of phagocytosis *in vitro*, that a very small amount of opsonin—even less than that which is present in a serum in which the index is very low—is quite sufficient to sensitize any bacteria that are likely to gain access to the tissues or blood. In our laboratory experiments the conditions are certainly much less favourable than they are in the living body; the leucocytes are certainly not in the same state of functional activity as they are in the body, and there is a *limited* supply of serum instead of a constant stream thereof. In spite of this, an enormous number of bacteria are



taken up, and in some cases digested, within a few minutes. In the body, of course, the action may go on for hours. The opsonin-leucocyte mechanism would *appear* far stronger than is necessary for the defence of the body. That it is not so indicates some fallacy in the conclusions to be derived from these experiments *in vitro*. We shall revert to this subject subsequently, and in the meantime be content with pointing out that where a very small amount of opsonin would appear sufficient for the resources of the body, but little importance can be attached to small fluctuations, or to a rise, *e.g.*, from 0.8 to 1.

The dread of a low opsonic index appears to have arisen on purely theoretical grounds, and the only direct research on the subject which seems to have been undertaken points rather in the other direction. According to Pfeiffer and Friedberger, guinea-pigs injected with bacterial vaccines (typhoid and cholera) do *not* thereby become hypersensitive to doses of living cultures given twelve or thirty-six hours afterwards; on the contrary, they have acquired an increased power of resistance, even after the shorter period. And a very remarkable fact was noticed: this increased resistance was not specific, since animals injected with heated typhoid bacilli survived a lethal dose of cholera as well as of typhoid. They conclude that the fear of a negative phase is exaggerated; and it must not be forgotten that the essence of the "opsonin therapy" consists in administering a dose of vaccine, in the first instance, while the index is low.

There is thus no direct proof that the period of the negative phase is coincident with the period of hypersensitiveness to infection. And when we compare it with the period of increased sensitiveness to toxins, we find that, whereas the negative phase comes on almost immediately, the hypersensitiveness to toxins or tuberculin, or anaphylaxis to serum, takes some days to develop.

Other theoretical interpretations of the undoubted good effects of vaccine therapy are possible. Thus, a very probable explanation is that it causes a local reaction in the form of an aseptic inflammatory process in the neighbourhood of the lesion, which, like the similar reaction caused by ultra-violet or X rays, has (in some way not yet understood) a curative effect. The nature of these "reactions" is considered subsequently; in the meantime it is sufficient to say that in the case of tubercle (and it is probably a general effect) an injection of dead bacilli, or of the

products thereof, causes a sharp rise in temperature and an inflammatory process around the tuberculous focus. If the dose of tuberculin be greatly reduced the local reaction takes place, but there is no rise of temperature. This is best seen when small doses of TR are used in the treatment of tuberculous iritis, in which the iris can often be seen to become injected after each dose; and I have observed the same reaction in a very marked form after the use of diluted old tuberculin in von Pirquet's reaction. In this case the dose absorbed must have been infinitesimal, since the temperature did not show the slightest sign of a rise.

Other possibilities are that the vaccine may cause a general tissue immunity, or that it may produce some degree of immunity on the part of the leucocytes, or may at least alter them in some way so that they are more able to perform their duties as phagocytes; and, of course, other antibodies, such as antiendotoxins, may be produced as a result of the injection, and of these the opsonic index affords us no estimate.

In reverting to the question of the nature and properties of the opsonins, the question of their thermo-stability first claims our attention. The results obtained by various observers are not quite in accord, and indicate very clearly that more than one substance may have the same action. The opsonin present in normal serum is in a high degree thermolabile. It is destroyed by heating to 55° C. for half to one hour; at 60° C. most disappears in five minutes, the rest more slowly, little being left in fifteen minutes. Wright and Reid, however, found that in cases of tuberculosis some of the opsonin is more thermostable, and whereas in heating a normal control to 60° C. for ten minutes reduces the opsonic index to almost nothing, the same proceeding may only lower the index of a tuberculous serum to 0.4 or so, though the indices of both samples were formerly the same. They suggested this as a means for the diagnosis of tubercle. Other observers have failed to corroborate their results, and they are certainly not true of all cases. Dean showed that in certain sera obtained by the high immunization of animals to certain bacteria (staphylococci, dysentery, and typhoid bacilli) there are substances which act as opsonins, and which are thermostable. His results have been corroborated for pneumococcic serum by Macdonald and Rosenau, by Muir and Martin, and many others. It is evident, therefore, that there is more than one substance

which can prepare bacteria for phagocytosis. There is a thermolabile substance which occurs in normal serum, and a thermostable one which is found in immune serum; and this latter also contains a thermolabile substance, since (as a rule) its index is lowered by heat. Thermostable opsonin occurs in minute traces in normal serum, since the index is never reduced quite to the level seen in a control specimen made with normal saline by heating to 60° C., and we need have no hesitation in recognizing it as a specific antibody. It will be convenient to deal with it first, and the question naturally arises, Is it amboceptor? In other words, Has amboceptor the power of preparing bacteria for phagocytosis in addition to sensitizing them to the action of complement? The two substances arise under the same conditions, and are identical in their power of resisting heat, faculty of combining with bacteria, and in their specificity. The second question arises, Assuming thermostable opsonin is amboceptor, is the action of complement also useful in preparing bacteria for phagocytosis, or does the process go on equally well without it? Now it is certain that complement is not necessary for the action of thermostable opsonin; otherwise it would only exert its action in a heated serum when subsequently activated by fresh serum, and this is not the case. If thermostable opsonin is amboceptor, therefore, it can exert its effects without the action of complement. But some experiments go to show that thermostable opsonin may be more potent when reactivated. Thus Crofton found an antistreptococcic serum might stimulate phagocytosis more when mixed with fresh human serum than with an equal amount of normal saline.

Similar results have been obtained more recently by Dean, who finds that the opsonic effect obtained by heated serum and normal serum may be greater than the sum of the two effects separately. The subject has been very carefully investigated by Chapin and Cowie, who were able to avoid the possibility of certain errors by performing their saturation experiments in a cold room, kept at 0° C. throughout the experiment. They found that a normal human serum treated with staphylococci at this temperature might have the whole of its opsonic power removed, and yet would still reactivate a heated serum—*i.e.*, the thermostable opsonin combines with bacteria at 0° C., and is probably amboceptor. They found that staphylococci treated with normal serum at 0° C. and then washed are slightly more susceptible to

phagocytosis than are normal ones, but the difference is not great. They are, however, much more easily opsonized by normal serum, or by serum that has had its amboceptor removed by treatment with staphylococci in the cold.

In other cases the conditions are more complex, for when a potent bacteriolytic serum is present, bacteriolysis may occur to such an extent as to diminish the number of organisms which can be taken up by the leucocytes. We then get the "reversed ratio" phenomenon described by Leishman and Dean. It is as follows: Under ordinary conditions the index falls greatly on heating, as has been shown. This is called the normal ratio. But in some of the potent sera obtained from highly immunized animals the opsonix index may apparently rise after heating to two or three times that of the raw serum. This Dean explains—and his explanation is an extremely rational one—by invoking the bacteriolytic action of the unheated serum. The number of bacteria in the emulsion is reduced, so that there are fewer for the leucocyte to take up; some that are not completely dissolved may lose their power of retaining stains and become invisible; bacteria partially acted on may be readily digested within the leucocyte, so that they are not counted; and, lastly, the dissolved bacteria may have a toxic effect on the leucocytes. The phenomenon of the reversed ratio may be taken as an argument in favour of the equivalence of thermostable opsonin and amboceptor.

The strongest argument, however, is derived from the experiments of Dean, who has shown that in different samples of immune sera there is a distinct parallelism between the two functions: when the serum is powerful as a bacteriolytic agent, when activated with a suitable complement, it is also powerful as an opsonin after heating. It must be admitted, of course, that a serum may be opsonic, but not bacteriolytic; but this is explicable on the assumption that much less of the substance is required to sensitize the bacterium to the attack of leucocytes than is necessary to render it soluble by complement. This has been confirmed by Neufeld and Bickel, who found that a very minute amount of hæmolytic serum, far less than would produce hæmolysis, would act as a hæmopsonin.

The opsonic index does not rise *pari passu* with the bacteriolytic power, but this is partly due to the fact that the criteria are

different in the two cases. We have already shown that increments in the amounts of opsonin cause smaller and smaller rises in the opsonic index as we proceed. There is, however, a much closer parallelism between the bacteriolytic power and the amount of thermostable opsonin present as shown by the degree of dilution. This is well shown (in the case of typhoid fever) by the chart given by Klien and inserted previously (Fig. 58).

To sum up: Amboceptor appears to have the power of sensitizing bacteria for phagocytosis, and this power appears to be increased by the concurrent action of complement. Further, there appears to be no sufficient evidence for the existence of a thermostable opsonin apart from amboceptor, as has been maintained by Neufeld and Hime.

(There is an additional possibility that the part of a thermostable opsonin may be enacted by agglutinin.

I believe that in the case of the hæmopsonins of normal human serum the substance is a thermostable agglutinin with a second thermolabile zymotoxic group. Natural hæmopsonic sera are, as far as I have seen, always powerful agglutinators of the red corpuscles which they opsonize, and when they are heated to 60° C. the opsonic power is destroyed, but the agglutinative faculty is unaltered.)

These facts may serve to explain the discordant results as to the presence of thermostable opsonins in the sera of tuberculous patients. It has been shown by Bruck that antibodies to the tubercle bacillus are not always or usually present in the blood of infected persons, and it is only when they are present that we should find a thermostable opsonin.

If thermostable opsonins resemble amboceptor in their properties, there is an equally close resemblance between thermolabile opsonin and complement. Each occurs in normal serum, and is destroyed by a short heating to 55° to 60° C. Are they the same?

The main fact against the theory of their identity is the specificity, partial though it may be, of the opsonins; for there is no reason to think that different bacteria are attacked by different complements, even if we accept the theory of the multiplicity of these bodies to the fullest degree. But we have already seen that the specificity of the opsonins is not complete, and that the

whole of the staphylococcic opsonin may be removed by the addition of sufficient amounts of tubercle bacilli.<sup>1</sup>

A second fact, closely allied and perhaps in reality identical with the foregoing, is the rise in a particular opsonin after an injection of a suitable vaccine, the others remaining constant. This rise cannot be accounted for (in my opinion, at least) by the appearance of small quantities of thermostable opsonin, since it may occur when this substance cannot be found in the serum.

On the other hand, there are very remarkable analogies between the two substances. In each there is the same difference of opinion as to whether it occurs in normal plasma, or is only developed when clotting and destruction of leucocytes occur. Wright and Douglas found the amount of opsonin present in serum and in citrated plasma exactly the same, whereas Briscoe found that very little phagocytosis took place when staphylococci were injected into a surviving heart in which no clotting took place. These divergencies are quite similar to those found by different investigators in the case of complement.

Again, it has been already shown that when a blood-clot contracts, the first serum which can be collected is poor in complement compared with that which follows, and that after a time the amount again diminishes. An exactly similar phenomenon may sometimes, though apparently not always, be demonstrated with opsonin (Henderson Smith). Hence an important practical point: the patient's blood should always be collected at the same time as the control in determinations of the opsonic index.

Thirdly, it has been shown by Levaditi that the aqueous humour of the rabbit contains no complement and but a trace of opsonin. But when the fluid which recollects after puncture was examined, it was found to be rich in both substances. He found a similar relation between the two substances in œdema fluid. As against these results we have to put the researches of Leding-

<sup>1</sup> Since the above was written Muir and Browning have adduced very definite evidence of a partial specificity in the case of the complements. They find that the bactericidal action of normal serum may be due to the direct action of complements, and that, on weakening normal serum by successive additions of dead bacteria, the first effect is a falling off in the bactericidal action as tested on that bacterium. Then the bactericidal action on the other bacteria is diminished, and with a larger addition the hæmolytic complement is absorbed. This indicates features exactly like the partial specificity seen in opsonins, and a similar absorption without the intervention of an immune body.

ham and Bulloch, who found that when the number of leucocytes in the blood was increased by injections of cinnamate of sodium, there was an increase in the complement, but not in the opsonin.

It may be pointed out that if opsonin and complement are the same, we must suppose that the opsonin test is the most delicate method of demonstrating this substance that we have, since phagocytosis may be facilitated by substances which in complements cannot be detected by ordinary tests. Further, we must assume that it unites with bacteria direct, and sensitizes them for phagocytosis without the intervention of amboceptor. There is no serious difficulty in accepting both suppositions.

Lastly, Muir has shown that the substances which have the power of absorbing complement (such as compounds of red blood-corpuscles and their amboceptor) also remove the thermolabile opsonin. We are forced, therefore, to the conclusion that complements may play the part of opsonins. But to do this we must necessarily broaden our ideas of the complements, and attribute to them some degree of specificity; otherwise the opsonic index of any given sample of serum as measured against a given control should be always the same, which, as we have already emphasized, is not the case (see footnote, p. 286).

These results suggest another train of ideas as to the rôle of bacteriolysis in immunity. We have already seen reason to believe that this is not of the greatest importance, and have found it difficult to think that so elaborate a mechanism should be of so little apparent use. May it not be that the complements are specially intended for use as opsonins, and that their action in bacteriolysis is a secondary one, and comparatively of less importance? This, of course, is a close approximation to Metchnikoff's views, but there is this difference: his cytase is a digestive ferment which, in the case of microcytase, is adapted to attack all sorts of bacterial proteid. But with the opsonins or complements we must assume that different molecules occur which have different combining affinities for the protoplasm of different bacteria, or, in other words, which differ slightly in their haptophore groups. Yet this difference is one in degree and not in kind, for they all have some power of uniting with all bacteria, and a great power of uniting with the bacterium-amboceptor combination.

On this theory the appearance of amboceptor will take on a new

significance, and we must regard this substance as a device for attaching *more* complements and more varieties of complement to an invading bacteria than can easily combine with it direct. In other words, we must regard the cytophile group of the amboceptor as being specific, whilst the complementophile group has the modified specificity which we attribute to the opsonins. The presence of amboceptor will therefore enable the bacterium to be prepared for phagocytosis by the concurrent action of many complements which otherwise would only be able to attack it with great difficulty.

And many facts, notably the liberation of endotoxin taking place when bacteriolysis occurs, would lead us to believe that this preparation for phagocytosis is the true function of amboceptor and complements, and that the appearance of the latter in excess is a comparatively rare phenomenon in disease, and when it occurs in enormous amounts (such as is seen in highly immunized animals) is an artificial phenomenon comparable with the enormous amounts of antitoxin seen in antitoxin-horses. Recovery from an attack of disease caused by *B. coli* may occur without the appearance of any amboceptor to *B. coli* demonstrable by ordinary tests; there may, nevertheless, be quite sufficient to act as a thermostable opsonin. We are far from denying that bacteriolysis ever occurs under natural conditions, but when there are plenty of leucocytes of sufficient functional activity, it is difficult to avoid the conclusion that they would ingest the bacteria when these were sensitized by complement alone, or complement and a little amboceptor, and before this latter substance had been developed in amount sufficient to cause bacteriolysis. This latter process may perhaps be the last line of defence, to be used only if the leucocytes are injured by the toxins or by the high temperature, or if they are present in insufficient numbers.

It has been pointed out already that there is some reason to think that, whilst complement and amboceptor can each sensitize for phagocytosis separately, they exert a more potent action when both are present.

As regards the *source* of opsonin, little is definitely known. If we regard the thermolabile opsonin as identical with complement, we shall regard it as probably derived from the polynuclear leucocytes, and this is corroborated by Levaditi's observations on the aqueous humour. Eyre has also shown that the amount of opsonin (to pneumococci) in the serum in pneumonia may be



roughly parallel with the number of leucocytes per cubic millimetre (Fig. 69).

This, however, was not corroborated by Bulloch and Ledingham in the case of the hyperleucocytosis caused by cinamate of soda. But it is highly doubtful whether leucocytes hurried prematurely from the bone-marrow, etc., are, as the result of the injection of chemical substances, as active functionally as those occurring normally in that situation; and this is corroborated

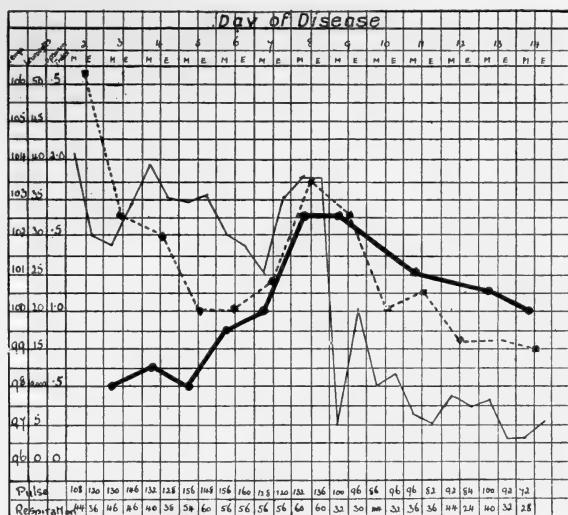


FIG. 69.—RELATION BETWEEN LEUCOCYTES, OPSONIC INDEX, AND TEMPERATURE IN A CASE OF PNEUMONIA. (Eyre.)

Dotted line=number of leucocytes per cubic millimetre; thick line=opsonic index; thin line=temperature.

by the fact that these observers found the leucocytes in question deficient in phagocytic powers. The point is one of some importance in connection with the lack of benefit which so often follows an artificial leucocytosis brought about for therapeutic purposes.

A few words on the subject of Metchnikoff's views on the opsonins may be added. He thinks that when bacteria gain access to the blood or tissues, the presence of opsonins or other preparatory substances is unnecessary, and the unaltered organisms can be attacked by the fresh and vigorous leucocytes. The

absence of phagocytosis *in vitro* in the absence of serum he attributes to a weakening and injury of the cells, due to the method by which they are prepared, and admits that these weakened and altered leucocytes will ingest bacteria more easily and more quickly if the latter have previously been prepared by the action of serum. He admits, however, that the opsonic index determines the defensive resources of the blood, and in doing so would appear to range himself definitely amongst the adherents of the opsonin theory. But there is no reason to think that washed leucocytes are weakened in respect of their phagocytic powers; they can take up enormous numbers of (opsonized) bacteria in a very short space of time, and it is difficult to believe that they could take up more in the living body; and if, as there is reason to think, phagocytosis is a physical process akin to agglutination, the functional activity of the leucocytes is a factor of little importance in phagocytosis, though essential for the other, equally necessary, phenomena of digestion and solution which take place subsequently. Metchnikoff finds that washed bacteria can take up large numbers of bacteria slowly, even in the absence of serum. This, however, proves nothing, since we have seen there is some reason to believe that opsonin may be formed from the leucocytes themselves. But, as a matter of fact, the increase in phagocytosis in preparations incubated for one or two hours as compared with those incubated for fifteen minutes is slight as compared with that consequent on the addition of serum.

The influence of *the source of the leucocytes* taking part in phagocytosis is not yet fully investigated, and there are no facts known at present which tend to show that those from an immunized animal have any special powers in this direction. Bulloch showed in a few cases that leucocytes from different sources would take up the same number of bacteria if used with the same opsonic sera. There are also observations tending to show that diseased or abnormal leucocytes—*e.g.*, those produced in excess as a result of the injection of certain substances, such as nuclein—are deficient in phagocytic activity.

In a very few cases some phenomena indicating an immunity, and consequent increased phagocytic power of the leucocytes, from an immune or infected person, as compared with the normal, have been noticed. This, of course, is quite in accordance with Metchnikoff's theoretical views. The examples are not numerous,

and the best is, perhaps, that given by Bassett-Smith, who found that in Malta fever the patient's leucocytes may be decidedly more potent than normal ones, when used in conjunction with the patient's serum, though, when normal serum is used, the difference may disappear. Thus :

					<i>Cocci per Leucocytes.</i>	
					23°0	46°0
Patient's serum	+	patient's leucocytes	+	emulsion of cocci	8°6	25°0
"	"	+ normal leucocytes	+	"	16°0	30°0
Normal serum	+	patient's leucocytes	+	"	19°0	29°0
"	"	+ normal leucocytes	+	"		

Rosenau has also brought forward evidence to show that leucocytes from cases of pneumonia have greater phagocytic powers than those from healthy persons, and are less easily killed by heat.

Much attention was attracted of late by Bail's theory of the *aggressins*. Bail found that if washed tubercle bacilli were injected in large amount into the peritoneum of guinea-pigs infected with tubercle, the animals died rapidly—*i.e.*, in eight hours or so.<sup>1</sup> There was a fluid exudate (containing lymphocytes) in the peritoneal cavity, and this exudate (centrifugalized to get rid of cells and bacteria) was found to have a remarkable action in increasing the virulence of young tubercle bacilli to normal animals. Thus, if a few cubic centimetres were injected together with the bacilli, death occurred in about twenty hours, instead of in some weeks. He found that this virulence was apparently due to an inhibitory effect which the fluid exerted on phagocytosis. When bacilli were injected into a normal animal *without* the exudate, many polynuclears appeared in the peritoneal fluid and many large mononuclear cells, and many of the bacilli were taken up; but when bacilli and exudate were injected, few cells other than lymphocytes were seen, and there was no phagocytosis.

These observations were confirmed and extended by Bail and others, and similar phenomena were found to occur in the case of numerous other organisms, if not in all. A very striking example was given by Weil in the case of the bacillus of chicken cholera, which is extremely virulent to rabbits, so that a millionth of a culture (containing perhaps but one bacillus) is certainly fatal. A minute trace was injected into the pleura, and the animal died in a few hours. Several cubic centimetres of turbid exudate, the

<sup>1</sup> This, of course, is equivalent to the tuberculin reaction in an extreme form.

cells of which had not taken up any bacteria, were collected, and were found to have a most potent effect in increasing the lethal action of the organism. This could not be tested on rabbits, since they were too susceptible, but in guinea-pigs it was found to lower considerably the lethal dose. A most interesting observation was made: A guinea-pig which had received a small dose of a culture of chicken cholera, and had apparently recovered completely, was injected eight days after with some of the exudate, and died of chicken cholera septicæmia, showing that the bacteria were but latent, and had been allowed to become virulent and active in virtue of the action of the exudate. Further, this fluid, when injected into rabbits, was found to immunize them against subsequent injections of the organism, even if mixed with the exudate, and so rendered more virulent.

To these substances Bail gave the name of aggressins, and considered them to be an entirely new type of specific substances formed by the organism, and having the power of raising its apparent virulence by checking phagocytosis and allowing the invading microbe to flourish without hindrance: thus, by means of the concurrent presence of its specific aggressin, an almost innocuous organism, such as *B. subtilis*, becomes extremely virulent. According to Bail and his followers, aggressins are only formed *in vivo*; but this is denied by others, who claim that a watery emulsion of certain bacteria has many at least of their peculiar characters.

Immunity due to the injection of aggressin is supposed to depend on the formation of a specific antibody, or anti-aggressin. It is produced very rapidly after the injection of the aggressin, and lasts several weeks or more, and is supposed to be due to the immediate neutralization of any aggressin which the bacterium may form *in vivo* by the anti-aggressin, so that phagocytosis is unchecked.

Aggressins are sharply specific, except perhaps in the case of those for *B. typhosis* and *B. coli*—*i.e.*, the injection of one aggressin will not prevent the phagocytosis of any species of bacterium other than that by the action of which it was prepared; hence they are not mere leucocyte poisons: they are thermolabile.

A substance which prevents phagocytosis may act on the leucocyte, the bacterium, or on the serum. The fact just described (that phagocytosis of a bacterium A can go on in the presence of an aggressin B) shows that the action of the aggressins is not on the leucocytes. Further, as Weil and Nikayama have shown, bacteria which have been acted on by their aggressins and the

latter removed by washing, are readily ingested. The action, therefore, must be on the serum—*i.e.*, aggressin must act as an anti-opsonin.

This leads us to Wassermann and Citron's explanation of the phenomena. They suppose aggressins to be simply solutions of the bacterial protoplasm which have the power of combining with the specific protective substances of the animal, and so disarming its methods of defence. In other words, they are solutions of endotoxin of feeble toxicity. This view is strongly supported—indeed, practically proved—by the researches of Doerr, who found that aggressins caused a precipitate when mixed with their specific immune sera, and that their presence might bring about an absorption of the complements, just as if they were free bacterial receptors. There are a few minor differences between aggressins prepared *in vivo* and those obtained from cultures *in vitro*, but not more than we might expect from the differences in their mode of production.

If aggressins are merely free molecules of bacterial protoplasm, we should expect them to combine with opsonins, just as do the bacteria themselves, and hence to act as anti-opsonins. And this supplies a striking proof of the specificity of the opsonins, for, as already stated, an aggressin of one organism (*e.g.*, *B. coli*) does not prevent the phagocytosis of another organism (*e.g.*, *B. subtilis*). This must apply to the thermolabile opsonin, or opsonin proper, since these experiments were made on normal animals.

The relationship between *virulence* and phagocytosis is an interesting one. As a general rule, it will be found, as shown by the extensive researches of Metchnikoff and his school, that there is an inverse ratio between the two: when an organism is virulent for an animal it will be ingested by the leucocytes to a very slight extent, and *vice versa*. This refers, of course, mainly to natural immunity, since in acquired immunity other factors, such as the action of bacteriolysins or antitoxins, may come in. There are, however, some exceptions. Thus, tubercle bacilli injected into the peritoneum of normal guinea-pigs are readily taken up by the phagocytes. We must assume in this case that an organism may be taken up whilst it is alive and uninjured, that it may be entirely indigestible by the leucocyte, and may continue to grow and multiply in its interior. This is also sometimes seen in acute infections: the common localization of the meningococcus in the polynuclear leucocytes is well known, and Andrewes has described

a case of general hæmic infection by this organism which ran a rapid fatal course in spite of all the organisms (as far as could be seen) being taken up by the leucocytes. In general, however, the law holds good, and where there is abundant leucocytosis the disease tends to recovery ; when there is little or none, to death.

As far as we know at present, the failure of phagocytosis which occurs with virulent bacteria is due to their deficient opsonization ; but whether this is because they require a large dose of opsonin before they can be ingested, or whether the opsonin cannot combine with them, has not yet been determined quite satisfactorily. It is this resistance which very virulent bacteria exert to phagocytosis which causes the very high indices seen in meningococcic infections. If the index is determined using the very virulent organisms recently isolated from a case of cerebro-spinal fever, very little, if any, opsonization and phagocytosis take place in the specimen in which normal serum is used, whereas a fair number are taken up when the serum from a patient is employed. If, however, the index be determined using an old laboratory culture, much more phagocytosis will be caused by normal serum, and the index will be nearer unity. The relation between virulence and lack of phagocytosis is discussed subsequently in the section on immunity to bacteria.

Lastly, many bacteria form toxins, of one sort or another, which prevent phagocytosis by a direct action on the leucocytes. It has been shown that tetanus spores and bacilli, when washed perfectly free of toxin, are quite innocuous to all animals, and are readily taken up by the phagocytes ; the presence of toxin, it may be in small amounts, by killing or injuring the leucocytes, allows the bacilli to grow in the tissues and elaborate more toxin. Similar facts probably occur in the case of diphtheria. We have already referred to the production of leucocidin by streptococci, and it is obvious that when this is formed in the tissues in large amount phagocytosis will be reduced or stopped altogether.

The nature of phagocytosis requires some discussion. We are, perhaps, rather too apt to be influenced by the readily observed phenomena of ingestion of bacteria, diatoms, etc., by amœbæ, and to assume that it is in all cases an active process on the part of the leucocytes, which are usually considered to approach their prey by active movements directed by positive chemotaxis, and to seize them by means of their pseudopodia. Chemotaxis does, of course, occur in the tissues, but it is clear that it does not take

place in the artificial conditions of opsonin estimations, where the bacteria are uniformly distributed throughout the fluid, and there is no reason why the leucocyte should be attracted in one direction rather than in another; and movement of leucocytes either does not occur at all or does so only to a very minute extent in saline solution. It takes place much more actively in unheated serum—a fact which gives some support to the theory of stimulins, previously mentioned, but not discussed. It is quite possible that all the facts related concerning opsonic action may be due to one or more substances which occur in the serum, and which have the power of stimulating the leucocyte, or of altering it in a manner to be discussed subsequently. The phenomena of the phagocytosis of sensitized bacteria in normal saline solution would, of course, be due to a liberation of this stimulin from its combination with the bacteria. This is known to occur, for sensitized bacteria will yield some opsonin on prolonged soaking in normal saline or heated serum; the fluid acquires opsonic properties, and the bacteria becomes insensitive to phagocytosis. As Sellards points out, the fact that unorganized bodies, such as carmine, particles of carbon, melanin, etc., are taken up more readily in the presence of fresh serum is somewhat in favour of this view. It is difficult to think that these substances are affected in a way similar to bacteria or other antigens when combined with their specific antibodies. There appears to be no crucial test for determining the point.

And there is some reason for thinking that the actual process of phagocytosis may be a physical one, akin to agglutination, and entirely independent of any movements or other vital processes on the part of the leucocytes. The chief evidence in favour of this view arises from the fact that phagocytosis may occur under conditions in which no movements of any sort take place. This was first pointed out by Ledingham in a series of important researches on the relation between temperature and opsonization. He showed that when a series of opsonin mixtures were incubated at temperatures varying between  $18^{\circ}$  and  $37^{\circ}$  C., the latter temperature brought about much more phagocytosis than the former; and, further, that at the latter point there was very little difference in the index between preparations incubated for fifteen or thirty minutes, while in the former there was a long latent period in which but little phagocytosis occurred. This he showed to be due to the fact that opsonin combines with bacteria but very

slowly at 18° C. and rapidly at 37° C. Provided the bacteria were sensitized at the latter, it mattered little or nothing whether the mixture were incubated at either temperature, and a very considerable amount of phagocytosis took place as low as 10° C. Now at this point no movements of any sort occur, and it is quite easy to satisfy oneself by actual observation under the microscope that bacteria opsonized at 37° C. may be taken up at a low temperature by bacteria which remain absolutely motionless during the process. This is even more easily observed by using a modification of a method recently introduced by Ponder, and of very great value in the direct observation of phagocytic and other phenomena. If a drop of blood be placed in a glass cell about 0.2 millimetre deep (such as is used in mounting diatoms, etc., in fluid), covered with a cover-glass, and incubated for fifteen minutes or so, both slide and cover-glass will be found to be dotted about with leucocytes which adhere so firmly that all the red corpuscles can be washed off with warm normal saline solutions, leaving the leucocytes adherent to the glass. If now the cell be filled with serum mixed with bacteria, and incubated at 37° C., or with bacteria thus opsonized and thoroughly washed, the process of phagocytosis can be readily watched, and is seen to take place at 18° C. or lower. Under these circumstances, no active movement or protrusion of pseudopodia takes place at all, and it is easy to watch a sensitized coccus being gradually attracted to and absorbed into the body of the leucocytes. The process strongly recalls the agglutination of bacteria. A coccus lying within a certain distance of the cell is seen, like the others, to be in active Brownian movement, and the appearances would suggest that it is slightly more easy for it to move towards the cell than away from it. It oscillates in all directions, but gradually approaches nearer and nearer the leucocyte, and is finally taken in. Similar phenomena can be seen (using a hot stage) when sensitized bacteria in an emulsion in normal saline solution are added to leucocyte films at 37° C.; and here also no movement, or but little, takes place. If, however, serum be added, there is usually some movement of the pseudopodia, but little or no locomotion from place to place.

It is not easy to determine whether phagocytosis may take place in dead leucocytes. I have not been able to detect it in leucocytes killed either by heat or cold, but Rosenau states that when leucocytes killed in the former way are mixed with opsonized



cocci, they collect round the cell, though they are not actually ingested, and this is confirmed by Sellards. Killed leucocytes probably undergo a sort of coagulation equivalent to rigor mortis, which would prevent the ingress of bacteria.

Sellards has shown that salts are as necessary for phagocytosis as for agglutination. The isotonic solution in which the leucocytes were suspended was 5.5 per cent. of saccharose; the bacteria were opsonized by fresh serum, washed thoroughly, and suspended in the same sugar solution. Little or no phagocytosis occurred, but it took place if salts were added. This, again, does not look like a vital process, but is quite analogous with agglutination, in which we have every reason to believe that the effect is due to an alteration of surface tension. So also with the action of serum in aiding the phagocytosis of substances such as carmine or carbon. We have only to suppose that some substance is occluded on the surface of the inert substance, the surface tension of which it alters in the same way as opsonin alters that of the bacteria.

The degree of opsonization is determined to some extent by the amount of salt present, and is found to be least (in the absence of serum) in a 1.2 per cent. solution; hence this strength of salt is used by some observers in opsonic determinations in order to reduce the amount of spontaneous phagocytosis as low as possible. Hamburger and Hekma have also shown that a minute trace of calcium chloride has a great influence in increasing the opsonic power of the serum (we have already seen that it aids the agglutination of cholera vibrios), and that the activity of the serum is increased by alkalis and diminished by acids. Chloride of potassium, unlike chloride of sodium, has also an unfavourable effect on the leucocytes.

If we push our investigations a little farther, we may perhaps be led to the belief that the amœboid movements and protrusion of pseudopodia which leucocytes display under suitable circumstances may themselves be effects of surface tension rather than strictly vital phenomena. Consider the case of the film of blood prepared by Ponder's method, or by the use of a glass cell, as recommended above. When this is incubated, large numbers of leucocytes appear both on the lower *and upper* surfaces. Now in the latter case the effect cannot be due to gravity, for the leucocytes are heavier than the serum. It would be too great a strain on the imagination to suppose the leucocytes capable of actual swimming movements through the blood (and it may be remarked that many

find their way to the top before coagulation occurs, though the process appears to continue after that), and the only alternative is a physical attraction between the glass and the leucocytes. This is a perfectly feasible explanation, and if it is true the next stage in the process would necessarily follow. This is the flattening out of the leucocytes, so that they form thin plaques of very much larger diameter than the same cells as seen in ordinary wet films. This is very difficult to explain as any vital effect, but it is exactly what we should expect to happen if the leucocyte (which, like all, or almost all, forms of living protoplasm, is to be regarded as a liquid) were pulled out under the influence of surface tension, just as a drop of liquid paraffin is stretched out into an infinitesimally thin film when dropped on the surface of water.

The bizarre forms which the leucocytes assume in a preparation made by Ponder's method, with long pseudopodia, are explicable on the assumption that, owing to irregularities in the cover-glass, the surface tension is not uniform in all directions, or that the protoplasm of the leucocyte is not of the same degree of viscosity throughout. Similar irregular protuberances can be produced in globules of oil or water by purely physical means, and Pauli goes so far as to say that "since the discovery of the amœboid movements of oil droplets, and the careful physical analysis of this process by Quinke, the formation of pseudopodia has been robbed of the characteristics of a specific life phenomenon, and later investigations have shown that it is governed in all its details by the laws of surface tension. The taking up of food and the process of defæcation in rhizopods can also be explained in the same way." The process of the ingestion of an opsonized bacterium suspended in serum at the body temperature, in which it is occasionally possible to see the protrusion and seizure of the organism by a long, slender, and flexible pseudopodium, is explicable as follows: Owing to the change of surface tension induced by the action of the opsonin on the bacterium, there is generated an attractive force which tends to draw the two together. The leucocyte, being fixed to the cover-glass like a sucker, does not move, but a small portion of its substance, being liquid or semi-liquid in consistency, is drawn out until it meets the bacterium, which is, of course, also attracted. The two meet, and then it will be found that the organism is firmly held in contact with the pseudopodium, so that it is not released even if the latter be carried to and fro by currents in the fluid.

The effects of surface tension may also be traced in some of the phenomena of inflammation, especially in the adhesion of the leucocytes to the vessel wall. It has been abundantly shown that this is due to an alteration in the latter, and it appears likely that this is simply due to a change in the tension developed at the surface between the endothelial lining and the serum, in virtue of which the former behaves like the glass in Ponder's method, attracting the leucocyte and causing it to adhere and flatten itself out. This extension, so as to offer as large a surface as possible, which is displayed by the leucocytes, and especially of the polynuclears, when they come into contact with a resistant surface, was noted long ago by Massart and Bordet, and in virtue of it they are able to make their way through the finest pores, even in compact bodies like bone and ivory. The remarkable deformation in shape which leucocytes undergo in acutely inflamed tissues is not usually appreciated. It was pointed out to me by Whitfield, and may often be seen at the edge of the sections where the fixation is perfect, provided the material has been placed in the fixing fluid immediately after its excision. The polynuclear leucocytes are often overlooked altogether, being pulled out into long strands of protoplasm containing nuclear filaments, giving the section a remarkable mossy appearance. This change in the surface tension of the vessels, lymph clefts, etc., probably plays a part of great importance in diapedesis. It is somewhat doubtful, however, whether it can afford a complete explanation of the phenomena of chemotaxis, in which a vital and apparently quasi-intelligent action appears probable.

It must not be imagined that the vitality of the leucocyte is to be regarded as unimportant in the consideration of phagocytosis as a means of defence. Here the process has only begun when the organism is ingested, and unless suitable digestive ferments are secreted, the bacterium dissolved, and the endotoxin absorbed or otherwise dealt with, the process is useless, or, by carrying bacteria out of the lesion to other parts of the body, may even be harmful.

## CHAPTER XI

### "REACTIONS" AND SIMILAR PHENOMENA

NOT long after the discovery of the tubercle bacillus Koch found that the effects of an inoculation of living cultures of the organism were quite different in normal and in tuberculous animals. If a normal animal is inoculated by scarification of the skin the wound soon heals, and in about a fortnight a hard nodule forms. This ulcerates, and remains an open ulcer until the animal dies. If a second inoculation be made after the first has run its course to the stage of ulceration, the process is profoundly modified. No nodule is formed at the site of the second inoculation, but the tissue round the first becomes hard, dark-coloured, and finally necrotic, and may be shed *en masse* and the lesion undergo complete cure. Koch found, further, that this change might be brought about by injections of dead cultures even after they had been boiled. He found, too, that a large dose of these killed cultures (which would cause nothing but local suppuration in normal animals) would kill a tuberculous guinea-pig in a short time—six to forty-eight hours—the symptoms being fever, acute inflammation, running on to necrosis, in the region of the tuberculous lesions, and in some cases generalization of the bacilli throughout the body. When very minute doses were used he found, on the contrary, that improvement might occur, and the tuberculous ulcer become cicatrized over.

This was made the basis of a method for the treatment of tubercle in man. But Koch found the use of killed cultures inconvenient, since the bacilli were but slowly absorbed, and might give rise to abscesses. He argued that the effect was evidently due to some soluble substance which diffused out of the bacilli, and after long research prepared the substance which is now so familiar as the *old tuberculin*. It is a solution in 40 to 50 per cent. glycerin of the soluble products of the tubercle bacillus, and is prepared by cultivating that organism for several weeks in

glycerinated veal broth in a thin layer, so that there is an abundant supply of oxygen. This culture is evaporated to one-tenth of its volume and filtered through a Chamberland filter. There are numerous slight modifications in the process of manufacture, but they are unimportant.

Old tuberculin is a syrupy brownish-yellow fluid, with a faint aromatic smell. It contains peptones and traces of other proteid bodies, but the nature of the substance on which its extraordinary power depends is quite unknown. It is in a sense to be regarded as a toxin of the tubercle bacillus, but it is not a true toxin, like those of diphtheria and tetanus, since it is practically non-toxic for healthy animals or for man. Its injection in large quantity may cause a slight febrile reaction, but not much more than a similar injection of peptones, etc., from any other source. It differs, also, in a marked degree from the exotoxins in that it is not destroyed by a temperature of 100°, or even of 120° C. It is dialyzable.

When injected into tuberculous animals it causes the same “reaction” as was produced by the living or dead culture, and this in very minute amount. A dose of 1 milligramme will cause a sharp reaction in a tuberculous patient, and, indeed, one-tenth of that amount will sometimes suffice. When we consider that the material consists mainly of the nutrient ingredients of the broth—Koch thought that the active principle might form 1 per cent. of the whole—its extraordinary potency is evident.

The phenomena of the “reaction” are as follows: There may be, but usually is not, some inflammatory œdema at the seat of injection. The temperature rises precipitously, often reaching 105° F. in a few hours, and falls almost as quickly. With this there are the usual symptoms of fever, malaise, shivering, etc. This is the *general reaction*. The *local reaction* occurs round the pre-existing tuberculous lesion, and is best seen in lupus, tuberculous ulcers, etc. Its severity depends upon the dose given. With a small dose there is a little redness and swelling and some mild inflammatory œdema, the whole lasting but a day or two. When it subsides the lesion often undergoes great improvement. After larger doses the local reaction is more marked, acute inflammation occurs, the tissues in and around the tuberculous foci undergo coagulation necrosis, and are cast off. When this takes place in the skin it may lead to complete cure, but in the internal organs it is a source of grave danger, often leading to dissemination of the bacilli and a consequent general infection. This occurred in the

early days of the use of the fluid, when it was hailed as a specific cure for the disease, and Koch's limitations of its use ignored. At present it is used as a method of diagnosis, and found to be of great value and devoid of danger if used with proper precautions. And there can be no doubt that the bad results obtained when the

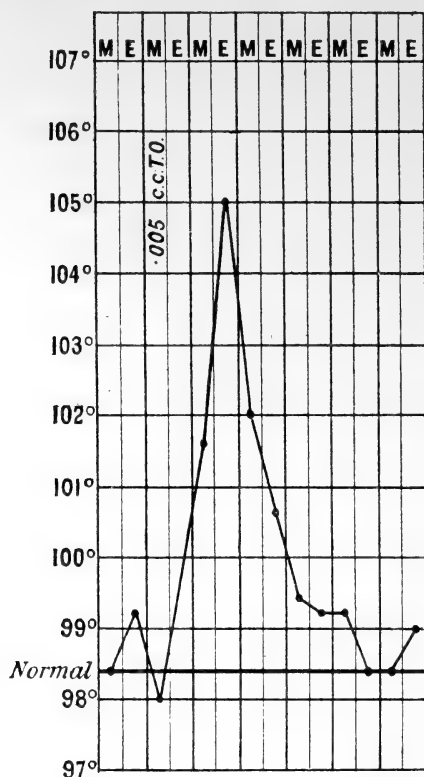


FIG. 70.—SEVERE TUBERCULIN REACTION IN A CASE OF BAZIN'S DISEASE.  
(Under Dr. Whitfield.)

potentialities of the substance were so little known have led to its being unjustly abandoned as a method of cure. Properly applied to suitable cases, it has proved of great value.

The reaction is a specific one, except that it is sometimes given in patients with syphilis, leprosy, or actinomycosis. This is unusual.

When patients are treated with gradually increasing doses of tuberculin they become partially immunized, so that no febrile

reaction is caused by large doses. Thus Wassermann records a case in which 300 milligrammes caused no reaction. The patient had been treated for a year, the dose being gradually increased. It appears, too, that by careful treatment of animals an antituberculin can be produced which has the power of inhibiting the effects of tuberculin in tuberculous animals. It has, however, little or no effect in the treatment of the disease—another proof that tuberculin is not the specific toxin.

Quite recently important modifications have been introduced in the diagnostic application of tuberculin. Von Pirquet's reaction, or the *cuti-reaction*, is elicited by placing a drop of tuberculin (undiluted or a 25 per cent. solution) on the skin, and performing scarification just as for an ordinary Jennerian vaccination. It is advisable to make a similar control scarification without using tuberculin in order that the lesions may be compared. The simple inoculation shows a little redness, which soon disappears. That made with tuberculin does the same if the patient is not tuberculous. If he is, a small red papule is formed, which increases for three or four days and disappears in a week or so.

Calmette's method, the *ophthalmo-reaction*, is obtained by instilling one or two drops of diluted tuberculin into the conjunctival sac. He recommends the use of old tuberculin which has been precipitated with absolute alcohol and redissolved in distilled water, as being less irritating and less likely to cause a pseudo-reaction in a non-tuberculous patient. The reaction in this case consists of a mild attack of conjunctivitis, lasting twenty-four hours, and accompanied by redness and swelling of the caruncle and a small amount of mucoid secretion. The reaction should be over in twenty-four hours, but in some cases undesirable results have occurred from a secondary infection with organisms capable of causing a more severe conjunctivitis or keratitis. For this reason the test should be used with care, if at all.

These tests are most applicable in children, since in adults the frequency of cured tubercle, also leading to hypersensitiveness, may lead to reactions where there is no clinical tubercle, or the patients may be more or less immune.

Reactions are given in other diseases, the most important being glanders. Mallein is a fluid obtained from cultures of *B. mallei* exactly as tuberculin is obtained from tubercle bacilli. It is non-toxic to normal animals, but it causes a febrile reaction in those infected by glanders, even if there is but a small latent lesion.

There is also a local reaction at the site of the lesion, though this is less marked than in tubercle. There is, however, a very marked production of inflammatory œdema at the site of the inoculation, and this furnishes the most certain test for the disease. A hard raised mass is formed, which increases in size for twenty-four hours or more, becoming as large as the palm of the hand, and persists some days. It often gradually travels down the neck (in the horse), as if under the action of gravity.

It will be noticed that an essential feature in these reactions is the *rapid* development of symptoms after an inoculation or injection in a subject already infected with the disease. Von Pirquet has brought forward other examples, which, if less dramatic in their course than Koch's phenomenon, are at least comparable with the cuti-reaction. Thus it has been noticed that the second (Jennerian) vaccination, practised some years after the first, runs a rapid course. Von Pirquet has shown that if a revaccination be made a few months after the first, the reaction takes place in twenty-four hours. It does not, of course, develop in the same way as a primary vaccination, but a small papule, often surrounded by an areola, makes its appearance, and lasts some two or three days.

Chantemesse has observed in typhoid fever phenomena similar to those seen in Calmette's ophthalmo-reaction in tubercle. He instils a single drop of typhoid "toxin" (obtained by cultivating typhoid bacilli in extract of spleen digested with pepsin), and finds that in normal persons there is but a little transient redness, whilst in typhoid patients there is redness, lachrymation, and the formation of a sero-fibrinous exudate. The process attains its maximum in six to twelve hours. He makes use of this reaction as a method of diagnosis.

The phenomena of the "negative phase," seen probably in all antibodies, but specially studied in connection with the opsonins, are probably similar in nature to these reactions, although the doses of vaccines given are usually so small that the clinical manifestations do not appear. Sometimes, however, this does happen. Thus Irons found that a dose of 500,000,000 dead gonococci caused no reaction in healthy persons; but if given to patients already suffering from a gonococcal infection, it produced fever, pains in the joints, and general malaise. In most cases the difference between the behaviour of a healthy and infected person or animal is traceable solely in the variations of the opsonic



index. When an ordinary dose of any vaccine is given to a healthy person the opsonic index undergoes but slight changes, and in particular there is no fall or negative phase. There may be a slight subsequent rise. When the same dose is given to a person infected with the same organism, the negative phase (perhaps preceded by a “false rise”) is most marked, and is followed by a positive rise, or sometimes by a series of rises and falls, gradually dying away like a wave. Similar phenomena can be produced in a healthy person, but here the dose must be much larger. Evidently, therefore, the presence of an infecting agent other than tubercle causes a condition of unstable equilibrium, in which the tissues react in a different manner to healthy ones. And the same condition of altered sensitiveness may persist for long after the disease or injection of a vaccine, so that a dose of dead bacilli that has but little action in health causes a great output of antibodies. This reaction appears to be a general one, occurring with bacteriolysins, agglutinins, etc.

Attempts have naturally been made to account for a phenomenon so remarkable as the tuberculin reaction, and the large number of explanations suggest that none is altogether satisfactory. Many of them do not call for notice.

Koch's explanation, which was put forward more as a working hypothesis than as an established fact, was this: The bacillus formed a toxin, which diffused outwards from the colonies in the tissues, and when in a sufficient state of concentration set up a coagulation - necrosis going on to caseation. In the zone of tissues just beyond this region the necrosis-producing substance is present, but not in a sufficient degree of concentration to kill the tissues. The injection of a little more of this substance—*i.e.*, of tuberculin—is sufficient to turn the scale, and a rapid increase of the necrosis takes place. He explains the beneficial effects of the treatment in this wise: The necrotic tissue does not form a suitable medium of growth for the tubercle bacillus (which is but rarely seen in caseous material), and the extension of the process may lead to the complete enclosure of the bacteria in dead and altered tissues, in which they are incapable of further growth.

This theory assumes that the substance which produces necrosis is identical with the active principle of tuberculin; but tuberculin in large doses will not produce necrosis in a healthy animal. It seems also to fail to account for the remarkable rise in the temperature, since it occurs in patients who are not febrile, as

we should expect them to be if tuberculin were diffusing from their lesions.

Ehrlich's views are quite similar to Koch's, and he regards the reaction as due to the effect of the tuberculin on tissues which are injured by it at the time of the injection, and in which a slight extra dose is sufficient to turn the scale.

Others have thought that the reaction is indicative of a hypersensitiveness of the patient to tuberculin, using the term in the sense in which we employed it in dealing with the toxins. This, of course, is true, but it scarcely seems a sufficient explanation in itself. We shall revert to the subject after giving an account of some most remarkable discoveries that have recently been made concerning this subject.

Marmorek holds—in all probability correctly—that tuberculin is not to be regarded as in any sense the true toxin of the tubercle bacillus. This is only formed when the organism is living parasitically in the tissues, or in artificial conditions bearing a very close approximation thereto, not in such a simple medium as plain broth. Tuberculin has this effect on a tuberculous animal: it stimulates the tubercle bacilli to a sudden and energetic production of toxin, which gives rise to the local reaction, and, passing into the vessels, to fever and its concomitant general phenomena. There is nothing inherently improbable in this suggestion, except that no reason is forthcoming as to the way in which tuberculin exerts this very remarkable action, but there is little direct evidence in its favour. The toxin which Marmorek claims to have produced by the application of this principle is so weak as not to be worth calling a toxin.

Wassermann and Brück point out that the extremely minute amount which must be present in the blood at a given time leads to the supposition that the tuberculin injected must leave the bloodstream and become concentrated in the region of the tuberculous focus. Thus, if a person with 5,000 c.c. of blood reacts to an injection of 1 milligramme of tuberculin, the dilution will be 1 : 5,000,000, and they find that this dilution injected directly into a tuberculous lesion gives absolutely no reaction. They then proceed to argue that this attraction of the tuberculin from the blood must be due to the presence in the tuberculous tissue of an antitoxin or anti-tuberculin. They investigated the presence or absence of this substance by means of the method of fixation of the complements. Extracts of tuberculous tissues, when mixed with tuberculin,

acquired the power of absorbing hæmolytic complements from fresh serum, and so of inhibiting the hæmolysis of sensitized red corpuscles. Extracts of normal organs had no such power.

This is made the basis for their theory of the reaction. The injected tuberculin circulates in the blood until it reaches the antituberculin present in the lesions. The two combine, and in doing so attract the complements which we must suppose to be free in the plasma. This fixation is supposed to be followed by cytolysis of the cells of the part. This accounts for the local reaction. In this solution of the tissue cells products of disintegration are set free, pass into the blood, and give rise to fever, causing the local reaction. Thus neither the local nor the general reaction is due to the direct toxic action of the tuberculin itself. In this the theory approaches somewhat to Marmorek's, and is in fundamental opposition to the older theories of “addition.”

Wassermann and Brück bring forward an important piece of evidence in favour of their theory by finding antituberculin present in the serum of patients who had been treated with increasing doses of tuberculin and had lost their power of reacting. In them the tuberculin injected would be immediately neutralized in the blood, and so never reach the lesion. The theory is ingenious, and may possibly turn out to be the correct one, but there are difficulties. Thus the authors find tuberculin as well as antituberculin in the diseased tissues, and it is difficult to see why the two do not neutralize one another. And we might also ask why no digestive phenomena should follow the union of the antituberculin and tuberculin in the blood of injected patients, and the subsequent absorption of the complements. Why should not the proteid molecules be digested, liberate their products, and produce fever? It would seem that the antituberculin present in the lesion must be in a state of fixation to the cells, or it must be carried away in the blood-stream, and this, according to Wassermann and Brück, rarely happens except after injections. But we do not know definitely of any such antitoxin, the nearest approach to it being a superabundance of suitable sessile receptors, which, if they occurred, might very well make their way into the extracts used in the test, and simulate an antitoxin. And if this were the case, there is no explanation why these receptors are not shed in the normal tuberculous process, but are after the use of tuberculin. It is difficult, too, to see why the presence of these abnormally numerous receptors might not be made the basis for a “theory of addition”

without invoking the aid of the complements. But the whole subject is theoretical to a degree, and needs, moreover, independent experimental verification.

Bail's researches on the aggressins have been referred to already. The application of his theory to Koch's phenomenon is obvious. According to him the endotoxins are only set at liberty when bacteriolysis occurs, not after phagocytosis. This is in all probability correct in most cases, though perhaps not in all. When, therefore, tubercle bacilli are injected into the peritoneal cavity of a normal guinea-pig and extensive phagocytosis occurs, there is little or no febrile reaction; but in the tuberculous animal the bacilli produce aggressins, which paralyze the phagocytes, and, when a second injection is made, the bacteria undergo rapid bacteriolysis, endotoxin is set free, and rapid death follows. Bail compares the results of the solution of large quantities of cholera bacilli in an immunized animal with what is seen after the injection of tubercle bacilli along with a small amount of the peritoneal exudate from a tuberculous animal. In each case there is extracellular bacteriolysis, and death in a few hours, obviously from the toxin set free.

This might account in a satisfactory way for Koch's phenomena when caused by the injection of cultures, but seems to fail utterly when applied to the tuberculin reaction; for tuberculin is neither an "aggressin" in Bail's sense, nor an endotoxin of the tubercle bacillus, and it cannot undergo bacteriolysis. The theory resembles that of Marmorek.

Von Pirquet's explanation of the early reaction after Jennerian vaccination calls for some notice, though it is not immediately applicable to Koch's phenomenon. It introduces some new and interesting conceptions. According to this author, the result of an infection is to alter the way in which an animal reacts subsequently to a second infection with the same organism. This he calls *allergia*. In some cases this may lead to hypersensitiveness, but in the majority it leads to a temporary immunity, followed by a condition in which the animal is no longer immune, but possesses the power of forming antibodies in the region of inoculation more quickly and easily than a normal one can do. When a second inoculation is made, the bacteriolysins present in the blood may be sufficient to destroy the bacteria introduced, setting free their toxins, which act locally and cause the early reaction. Or this may be delayed until local antibodies are formed. This occurs

more quickly than in the normal person. It leads to an early development of the specific lesion of vaccinia.

The essential point of this theory is that an infection from which recovery has taken place may lead to an alteration of the facilities with which antibodies may be formed, which alteration persists for a long time.

It seems desirable here to make a further reference to the subject already mentioned briefly as "hypersensitiveness to toxins," but now more generally termed anaphylaxis—*i.e.*, the opposite of prophylaxis. The term was introduced by Richet, who studied especially the poison of the actiniæ, which he found to be extremely powerful, the lethal dose being about .009 gramme per kilo of body-weight. He found that a non-lethal dose increased the susceptibility of the animal to a second injection, and that this hypersensitiveness might last as long as six months after the first injection. This, of course, is quite similar to the phenomena we have described in connection with diphtheria and tetanus, which renders it so difficult to immunize small animals to these substances, and which is the cause of much danger in the early stages of antitoxin formation in the higher animals. Richet has also studied the poison formed by the common mussel, which he calls "mytilo-congestine," and finds exactly similar facts; indeed, it is probable that it is a *general* phenomenon of all the poisons which can act as antigens. In the case of mytilo-congestine the measure of the hypersensitiveness is simple, since one of the most constant symptoms of its action is vomiting, which occurs almost as soon as the injection is made. He finds that in an animal which has previously been injected the emetizing dose is from a tenth to a quarter of the amount originally necessary. Richet has elaborated a theory to account for this phenomenon, and for anaphylaxis in general. He holds that the condition is due to the presence in the blood of a toxogenic substance, which gives rise to a poison after reacting with the mytilo-congestine injected. This toxogenic substance is not formed immediately, for Richet does not find hypersensitiveness to come on for five or six days, and it persists for some fifty days, that being the average duration of the state. He holds that the animal produces antitoxin also, but more slowly. When the toxogenic substance has disappeared the antitoxin remains, and the animal is immune. The main evidence in favour of this theory is the fact that the serum of an anaphylactic animal will produce a similar condition in a second animal. Currie has

enunciated a theory very like this in regard to serum anaphylaxis, to be described shortly.

Other theories might be cited, but there is only one which gives an explanation which is at all satisfactory without introducing many unproved suggestions. It was introduced quite recently by Goodman, and proceeds on lines somewhat similar to those we followed when dealing with the question of immunity to toxins. The cells of the body may be classified into three groups: (1) The nerve cells essential to life, and with a high degree of affinity for toxin; (2) cells not essential to life, but with a higher degree of affinity for toxin than the nerve cells possess; and (3) inert cells without susceptibility to toxin. If a dose of toxin be injected, the second class of cell will have its receptors satisfied first, and any toxin which is left over will then attack the nerve cells, which we assume to be the only region where it will do harm. A lethal dose of toxin, therefore, is the amount which will satisfy the receptors of the second group of cells and leave enough toxin to injure the nerve cells sufficiently to cause death. Now if a first injection just sufficient to combine with the receptors of Group 2 were given, a very small additional amount would be sufficient to cause death, since it would go straight to the nerve centres. So far the theory is unsatisfactory, since it is simply a theory of summation, and the total amount necessary to cause death, if given in divided doses, should together form the amount necessary if given in one dose, which is very far from being the case. We have seen that  $\frac{1}{400}$  of the "lethal dose" of tetanus toxin may cause death if given in divided doses. To account for this Goodman supposes that the toxin which combines with the non-essential cells may cause a sort of spreading necrosis of the receptors of the latter, or may interfere with their nutrition; in either case more of these receptors may be destroyed than the toxin actually combines with. If we can imagine one molecule of toxin destroying ten receptors, the animal would become as susceptible as if ten times the dose were given at once. Put in another way, if it takes  $x$  molecules of toxin to satisfy the receptors of non-essential cells, and  $y$  molecules to combine with those of the central nervous system and kill, then if in the sensitizing dose each molecule of toxin destroys ten receptors, the lethal amount necessary for a second dose would be but  $\frac{x}{10} + a$ .  $x$  we must suppose much larger than  $a$ .

He compares this process with the injury to the excretory

organs which often follows the action of poison. The kidneys, etc., excrete the substance, but in doing so are injured, and a smaller dose of the poison may now produce a great effect, since it cannot readily be eliminated.

The main objection to this theory is that it is difficult to imagine such a *selective* destruction of the receptors as seems necessary to account for the fact that the hypersensitiveness is specific. We should expect the creeping necrosis or interference with nutrition to act more generally, so that an animal highly sensitized to one toxin would show some degree of sensitiveness to others. It seems also inadequate to explain the facts of serum anaphylaxis, which will now be described, since here the animal is sensitized with minute amounts of a substance which causes no toxic symptoms in comparatively enormous doses in a normal animal. Here the anaphylaxis appears to be the production of a new sensitiveness rather than the exaltation of one previously existing.

There are two of these phenomena of hypersensitiveness to serum—Arthus' phenomenon and Theobald Smith's phenomenon, both of which are referred to as "serum anaphylaxis." The latter is the more important.

Arthus' phenomenon appears when a guinea-pig receives several injections, at intervals of a few days, of normal horse serum, a substance which in itself is scarcely more toxic than normal solution. After a few such inoculations the animal becomes hypersensitive, or *anaphylactized*, and after another injection an cedematous mass, an aseptic abscess, or an area of necrosis, appears at the site of a new inoculation, which need not be in a region in which a previous injection has been made; the alteration is a general, and not a local, one. After several of these injections the animal becomes cachectic, and dies after several weeks. An animal thus sensitized will die rapidly after the injection of 2 c.c. of serum into the veins.

It should be noticed that these results are not due to the accumulation of the horse serum in the system, since they may be brought about by the injection in divided doses of an amount which an animal can stand with impunity if given in a single dose.

Theobald Smith's phenomenon occurs when an animal has been sensitized by a very small injection of horse serum ( $\frac{1}{100}$  c.c., or even as little as  $\frac{1}{100000}$  c.c., an almost inconceivably small

amount to produce so great an effect), and kept for a fortnight or more. If then a second injection of a larger amount of the same serum be made ( $\frac{1}{10}$  c.c. or more, the usual testing dose being 5 c.c.), the animal develops a series of remarkable symptoms, the most noteworthy being respiratory failure, paralysis, and clonic spasms. Symptoms usually appear within ten minutes, and death occurs within an hour. Death does not always follow. The less sensitive the animal, the later the development of symptoms (which in highly sensitive animals come on within ten minutes), and the greater the chance of survival. The process evidently affects the nervous system in a very special way, and the heart may continue to beat for an hour after death. In some cases, but not in all, there are definite hæmorrhagic lesions present; they usually occur in the stomach, less frequently in the cæcum, lungs, spleen, adrenals, or other parts.

The phenomena had often been seen in the process of testing diphtheria and other antitoxins for the presence of free toxin, in which several cubic centimetres of the serum are injected intraperitoneally into guinea-pigs. Animals that have been previously used for the standardization of the antitoxin are often employed, and as these have received minute doses of the latter substance they may be hypersensitive. The phenomenon is a familiar one, but it is only recently that its true method of origin has been apparent. It has no connection with the antitoxin as such, and the same phenomena of hypersensitiveness may be produced by means of egg-albumin.

The action is to a certain extent a specific one. An animal sensitized with horse serum is less susceptible to the serum of the cow, pig, sheep, etc., than to that of the horse. It may show symptoms after the injection of one of these heterologous sera, but usually recovers. And the same is true for an animal sensitized by small doses of another sera. Symptoms are not usually produced by horse serum, and if they are, are not fatal. Animals can be sensitized by feeding with horse serum or with horseflesh. Rosenau and Anderson thought that children might be sensitized in this way, and so develop toxic symptoms after the use of antitoxin, but abandoned the idea.

Otto and Rosenau and Anderson thought that small doses were necessary for the production of this form of hypersensitiveness, large ones appearing to bring about immunity; but Gay and Southard show that large doses simply delay the incubation



period. After an injection of  $\frac{1}{100}$  c.c. or  $\frac{1}{250}$  c.c. the animal is hypersensitive in a fortnight or less, whereas after a dose of 8 c.c. the sensitiveness does not reach its maximum for some forty-five days. The duration of this anaphylaxis is not exactly determined, but it certainly lasts several months.

Gay and Southard further found that during the period of insensitiveness which follows a large dose the animal actually contains the substance which acts as a sensitizing agent. Thus a guinea-pig which had received (in divided doses) 17 c.c. of normal horse serum was bled fourteen days after the last dose: 1.5 c.c. of its serum was found to sensitize a normal guinea-pig, so that it died in ninety minutes after an injection of normal horse serum. (Rosenau and Anderson had already found that the young of sensitized animals are also sensitive.) Further, Gay and Southard found the sensitizing substance present in the blood of sensitized animals. Thus a guinea-pig received  $\frac{1}{100}$  c.c. of horse serum, and after twenty-nine days was bled, and 1.5 c.c. found to sensitize another animal. The first pig was tested and found to be sensitive.

These discoveries are sufficiently astonishing, and there appears to be no satisfactory explanation for them. The period of incubation would suggest that we are dealing with antibodies of some sort, but there is no evidence to show that the precipitins play any part in the process. An animal may be highly sensitive when no precipitin can be demonstrated in its serum.

Gay and Southard think that the hypersensitiveness depends on the presence of a substance which occurs in horse serum, and which they distinguish by the name anaphylactin.<sup>1</sup> This they do not consider to be the same as the toxic ingredient of the serum, for this reason: a sensitized animal will not develop symptoms after the injection of blood from an animal in the refractory stage, though this, as we have seen, was sufficient to sensitize it. (The injections were made into a vein, the sensitive animal reacting to very minute doses administered by this channel.) They failed to find any proof of the formation of antibodies in the animal during the refractory stage. They regard the reaction as being one of the cells of the sensitized animal, and as being due to a heightened power of absorption of the "toxic substance" on the part of cells which have been exposed for a certain period to the action of the anaphylactic substance. The action of this toxic substance

<sup>1</sup> Analogous to Richet's toxogenic substance.

appears to be to make the fatty constituents of the cells flow rapidly together. This occurs especially in the endothelium of the capillaries, and leads to hæmorrhages.

This can hardly be regarded as a full explanation, and fails, moreover, to explain a most remarkable fact discovered by Besredka: that sensitized animals do not react to the injection of horse serum if previously anæsthetized with ether. No theory that has yet been suggested will explain all the extraordinary phenomena connected with this subject, and no good purpose would be served by discussing others that have been brought forward. We seem to be on the eve of a series of discoveries that may prove to have as great an effect on our ideas of immunity and cell nutrition as the discovery of the antibodies themselves, and until the facts are better known hypotheses seem out of place.

We may, however, be permitted to point out that it is quite possible that the tuberculin reaction may be a phenomenon of exactly the same order as the serum anaphylaxis of the guinea-pig. Tuberculin is in itself non-toxic for a normal animal, just as is serum, but we may imagine that the tubercle bacilli in the lesions produce it in small amounts until the animal becomes anaphylactized, in which case it will react violently to a small injection. The wasting and fever which accompany the evolution of the disease may be phenomena akin to the phenomenon of Arthus, and simply indicate the reaction of a hypersensitive animal to repeated small doses of a non-toxic substance. If this is the case we may regard tuberculin as being, after all, the true toxin of the disease, though non-toxic to animals unless the processes of tissue nutrition and metabolism have been profoundly modified by the prolonged action of minute amounts of the same substance. That a reaction does not occur in a normal person after two injections of tuberculin does not surprise us, for the active principle is (as shown by the fact that it dialyzes) of small molecule, and may be eliminated more quickly than the large-moleculed toxic ingredient of horse serum, so that for hypersensitiveness to occur it may be necessary to have a continued stream of minute amounts from a lesion in the tissues. It may be pointed out, however, that occasionally a local reaction may be seen round the spots at which tuberculin has been previously injected, suggesting that the tissues in this region may be anaphylactic.

There appears to be no connection between this phenomenon of

serum anaphylaxis and the few cases that have occurred of sudden death after the injection of antitoxin, since these have usually (not invariably) occurred after the first dose. In some cases, if not in all, they have happened in children the subjects of the lymphatic diathesis, who are subject to sudden death on very slight provocation. And the remarkable lesions recorded by Gay and Southard have never been seen after death from a single dose of antitoxin; nor do the extraordinary symptoms develop.

This seems the most suitable place in which to discuss the "serum disease," or series of unpleasant though transient phenomena which may occur after the use of antitoxin, though it must not be considered that it is necessarily a phenomenon having any connection with those that have been already described.

The symptoms have been carefully analyzed by von Pirquet, and the following account is based very largely on his observations. The most constant symptom is fever, which is usually remittent in type. Next in frequency is a rash, which may be general or confined to the vicinity of the region at which the injection was made. The type of the rash varies in different cases, but all forms are associated with the most unpleasant symptom of the serum disease—namely, severe itching. The types of rashes are those included under the term "erythema multiforme." The severest, which is associated with the greatest degree of fever, is the morbilliform; the scarlatiniform is associated with less; and the urticarial rash, which is the commonest, is usually accompanied with but little elevation of temperature.

There is usually enlargement of the lymphatic glands corresponding to the region where the injection was administered, and this enlargement may be the first sign of the onset and of the cure of the disease. The pains in the joints often form one of the most unpleasant of the symptoms, and the articulations usually affected are the metacarpo-phalangeal, wrist, and knee, in that order of frequency. Albuminuria, not going on to nephritis, is occasionally present, and slight œdema is common. The severity of the disease is roughly proportioned to the amount of serum injected.

According to von Pirquet and Schick the leucocytes are increased in number until the symptoms develop, when a rapid fall (due chiefly to a decrease in the polynuclears) takes place.

The disease is painful, but not dangerous, complete recovery invariably occurring. The few cases of sudden death that have been recorded as taking place after an injection of serum appear

to be quite different in nature, and in many cases were dependent on the "status lymphaticus," or "thymicus," a condition in which a slight disturbance of any kind may cause sudden death.

In general terms, the severity and frequency of the serum disease are proportionate to the amount of serum given—the larger the dose, the greater the likelihood of its development and the more severe the disease. There are, however, some exceptions: (1) Certain samples of serum appear more potent in this respect than others. The purified diphtheria antitoxin prepared by Gibson (which consists of a solution of globulin) appears to reduce the occurrence of the disease to a minimum. (2) The serum disease is more likely to occur when a second injection is given at an interval of three or four weeks or longer after the first.

Under ordinary circumstances the disease manifests itself after an interval of eight to twelve days, or sometimes longer, after the injections. This period is insignificant, since it approximates closely to the period of maximum development of most of the antibodies after an injection of an antigen. Hence it was suggested (by Hamburger and others) that the symptoms might be due to the development of a precipitin, which, by combining with the unaltered horse serum still present in the patient's blood, might cause the production of small precipitates in the circulation. These, being deposited in the minute capillaries of the skin, joints, etc., might act as emboli, and cause the characteristic symptoms. Now the blood of a patient who has been treated with serum is frequently found to contain precipitin after a week or ten days, so that the possibility of this explanation is obvious. The phenomenon of the accelerated reaction also appears to lend it support. A patient who has been injected with horse serum may be presumed to have precipitin persisting in his system for some unknown but possibly lengthy period afterward, and on the injection of more horse serum (its antigen) might cause a precipitate which would lead to an immediate or accelerated development of the serum disease. It is found in practice that this immediate reaction does occur, but is comparatively rare. In ninety cases where a second injection was given the disease was developed within six hours in nine; in thirty-nine others it was produced within a period varying from nineteen hours to five days (Goodall). The "immediate effect" described by Goodall differs somewhat

from the usual “accelerated effect” occurring in patients injected with serum for the second time. The symptoms are rigor, pyrexia, vomiting, rash, and collapse, whereas the effects which develop in a day or two consist of rash, joint pains, and pyrexia.

The precipitation theory is now generally abandoned, since further investigation shows that there is no close parallelism between the occurrence of the disease and the presence of precipitins in the serum. Thus it is often found that the symptoms are present when not the slightest trace of antihorse precipitin is demonstrable. On the other hand, precipitins may occur when the disease does not develop, though this is a fact of less importance, since it is necessary, on this theory, that the precipitin and the unaltered horse serum should be present simultaneously, and this latter fact is usually impossible of proof. But Widal and Rostane have brought forward very strong negative evidence in showing that the disease does not necessarily occur after the intravenous injection of a powerful antihuman precipitating serum in man.

These arguments, though strong, cannot be regarded as entirely conclusive. As regards the absence of the precipitin in some cases of serum disease, it may be pointed out (*a*) that it may have all been removed in the form of precipitum when the sample was collected, and (*b*) that the demonstration *in vitro* of a minute amount of precipitin is by no means easy, and requires an appropriate correlation between the precipitating and normal sera, or the phenomenon may be missed. Further—and this applies to the negative experiments of Widal and Rostane—we do not know exactly what conditions are necessary to the union of precipitin and antigen to form an insoluble precipitate, and whether these are always present *in vivo*. Some experiments, it is true, appear to show that the combination never occurs in the animal body, but they are inconclusive, and the supposition is in the highest degree unlikely. It appears probable that precipitation, like agglutination, depends on the presence of certain salts, as well as on that of the antibody and antigen, and it may be that these are sometimes absent or not present in an available form. In this connection we may quote the researches of Netter, which are of great practical importance. He showed that the administration of calcium lactate at the time of the injection, and for a day or two after, caused a great diminution in the number of cases of the serum disease. His results have been generally substantiated,

and, in addition, the drug has been found to be of decided therapeutic value. Hence it appears that a diminished calcium content of the patient's blood at the time of the injection may be of some importance in bringing about the conditions necessary for the development of the disease. If this is the case, it might account for the symptoms in some cases and not in others, although precipitin might be produced in both. And there are probably other factors which are at present unsuspected, or the use of the calcium salt would be efficacious in all cases.

The alternative theory is, of course, that the patient gradually becomes hypersensitive to the serum, and that the grade of hypersensitiveness necessary for a reaction is reached before the serum is eliminated from the blood, or, in the case of an immediate or accelerated reaction, has not passed off before the second injection is given. It would make it a phenomenon analogous to those of Koch or Theobald Smith.

A good argument in favour of this theory is the long interval which may occur between the first injection and the second in the case of an accelerated reaction. This may be a year or more—a very long period for antibodies to persist in the blood after a single injection of antigen.

## CHAPTER XII

### COLLOIDAL THEORY OF ANTIBODIES

THE fact that antigens and their antibodies are, as far as is known at present, exclusively colloid in chemical character renders it advisable to glance briefly at some of the main facts and theories concerning these bodies and their reactions. They are substances of the greatest possible interest to the biologist, since the living tissues and the fluids of the living animal are, without exception, colloids and colloidal solutions; protoplasm, cell nuclei, etc., being mixtures of colloids in a jelly-like form, whilst the fluid part of blood-lymph, etc., is a colloidal solution. It is necessary to remember this, since the reactions of colloids and of crystalloids in presence of colloid appear to follow laws which are different from those governing the reactions of crystalloids alone, and we may doubt whether these latter ever take place in the living body. Colloids are not necessarily organic bodies, since metals such as gold and silver may be obtained in the colloidal state, as well as many metallic salts, such as ferric oxide, silicic acid, etc. These simple colloids appear to be governed by laws similar to those concerned in the reactions of the organic colloids formed by the action of vital processes.

The chief features which distinguish colloids from crystalloids are—(1) that they do not undergo dissociation into their ions when dissolved in water; and (2) that this so-called solution is not a true one, but merely a suspension of unaltered molecules or groups of molecules. These two characters are probably fundamentally the same. A colloid solution, therefore, is merely a very fine emulsion or suspension of particles of the substance, and Siedentopf and Zsigmondy have demonstrated a method by which these particles, though infinitely small as compared with the most minute bacteria, can be rendered visible and their numbers estimated. The method is simple enough in theory, though the actual arrangements are somewhat complicated. A powerful beam of light is passed trans-

versely through a true solution placed in the field of a powerful microscope, the ray passing at right angles to the optical axis. No light enters the lens, and the whole field remains in darkness. If, however, a colloid solution be similarly treated, these particles will reflect the light in every direction, and some rays will enter the lens. The result is that the particles are seen as minute luminous points on a dark background. The process is analogous with the examination of the stars with a telescope, which, no matter how powerful, never shows the star as a disc; but, merely by collecting more light, renders it brighter, and shows stars so small as to be invisible to the naked eye.

The particles in question are so small that they are prevented from falling to the bottom of the fluid by friction; if, however, they clump together, the particles become heavier, whilst the surface which they expose to the fluid does not increase at so great a rate, until at last aggregates of molecules are formed, which fall more or less rapidly. This is what takes place in coagulation of a proteid, whether brought about by heat or by the addition of electrolytes, etc. The factors which inhibit and which cause this clumping of molecules thus come to be of vital importance. The force which tends to cause this clumping is probably surface tension (Hardy, Bredig, Perrin), which is developed at the junction of the molecule and the water, and which, as explained in the section dealing with agglutination, tends to draw together any particles within a certain distance of one another, so that the surface may become as small as possible. This, of course, is not a peculiarity of colloidal solutions, but occurs in all emulsions of insoluble particles. Surface tension, therefore, is constantly tending to make the particles of colloid in a solution approach one another, form aggregates, and so cause precipitation or coagulation.

This action is counterbalanced in a stable solution by a force of electrical repulsion. The colloid particles all carry a feeble charge of positive or negative electricity, and therefore tend to repel one another. The existence of this charge and its nature can be shown by passing an electrical current through the fluid. Under these circumstances the colloids do not undergo dissociation with passage of the + ions to the - pole, and *vice versa*, like the electrolytes; instead, the molecules pass bodily to one or other pole, according to the sign of the electric charge they carry. Thus Field and Teague find that antitoxin is carried towards the cathode,



and must therefore carry a positive charge. Further researches show that absolutely pure colloids, free from all traces of electrolytes, carry no charge at all, and are not conveyed by an electrical current. The charge depends upon the nature of the ions present: traces of acid and of acid salts give it a positive charge, whilst alkalis and alkaline salts do the reverse (Pauli).

The process of coagulation of proteids, therefore, must depend upon the neutralization of this electrical charge, and this can be accomplished either by electrolytes or by colloids. Non-electrolytes (*i.e.*, substances which do not split up into electrified ions in solution) do not bring about coagulation in this way. Many of them, such as sugar and urea, are inert; others, such as alcohol, act in an entirely different manner. The precipitation of an albuminous solution by means of a strong acid takes place thus: The negatively-charged particles attract to themselves the positively-charged hydrogen ions; their charge is now neutralized, and the force of attraction due to their surface tension is no longer counter-balanced by an electrical repulsion; the particles are drawn together, form larger and larger aggregates, and finally cohere into masses so large as to come under the influence of gravity, when precipitation takes place. The coagulation of albumin by alcohol is due to the fact that proteids are not soluble in that fluid, so that when it is added to a watery solution of proteid, the water is withdrawn and the particles brought so close together that surface tension comes into play, and makes them coalesce into aggregates. The truth of this explanation appears from Pauli's observation that when no electrolytes are present alcohol acts very readily as a precipitant—there is no electrical repulsion between the particles. But if a little acid or alkali be added, and the molecules be thus given a mutually repellent electrical charge, the precipitation is inhibited or entirely prevented. Colloids can also be precipitated by colloids as well as by electrolytes, but in this case they must be of opposite sign. Thus, in testing for albumin in urine by means of acetic acid and ferrocyanide of potash, the addition of the acid insures that the particles shall acquire a positive charge (if they have it not already), which is then neutralized by the colloidal ferrocyanic acid of negative sign. Numerous other examples might be quoted.

This taking up of substances by colloidal particles is an example of the phenomenon known as *adsorption*. This process is difficult of definition, but in general terms we may regard it as a combina-

tion between two substances dependent on physical attraction rather than on chemical affinity, and taking place in very variable ratios, rather than in simple ones dependent on combinations of atoms or molecules, such as occurs in true chemical union. But it must be admitted that there is no absolutely sharp line of demarcation between the two classes of phenomena. In most cases the two substances entering into the phenomenon of adsorption exist as such side by side in the compound, which is to be regarded rather as an intimate admixture of the two than as an entirely new substance. The question arises as to whether the union between antigen and antibody is not really a process of adsorption, and in the attempt to solve this problem several very startling analogies with colloidal adsorption have been discovered. It will be convenient to discuss some of these, and then to give an account of the analogies met with in the chemistry of the colloids.

Bordet has shown that the amount of hæmolytic immune body which can be taken up by a given volume of corpuscles varies according to whether the corpuscles be added at the same time or in successive small portions. Thus, in one example 0.4 c.c. of a hæmolytic serum dissolved 0.5 c.c. of corpuscles if added at once. But if 0.2 c.c. of corpuscles were added first, and then successive amounts of 0.1 c.c. put in, no solution took place after that of the first portion added. This he explains, as we have already seen, by invoking a physical process of the nature of adsorption, comparing it with the adsorption of a dye by filter-paper. Other explanations may be possible, but an exactly similar phenomenon may be seen in the mutual adsorption of colloids of opposite sign. Thus, if to a given amount of solution of an electro-positive colloid an amount of solution of an electro-negative colloid exactly sufficient for neutralization of the opposite charges be added, the result will be the immediate commencement of the process of agglutination, which will go on until all the colloids are precipitated. But if a small amount of the second colloid be added to the same volume of the other, a different state of affairs is brought about. New aggregates of the two are formed, which are not necessarily neutral in reaction, and experience shows that the conditions are now less favourable to precipitation, which may require much more of the second colloid for its complete accomplishment, the intermediate bodies being apparently less easily precipitated than the unaltered colloid. Similar phenomena may also be seen in the

action of agglutinin or bacteria. Here also the solid particles may take up much more antibody than is necessary for agglutination to be induced if the serum be added all at once. It may be pointed out that this has a practical value in the estimation of the degree of agglutinating action as determined by the degree of dilution in which the action will take place. It is not proper to make a strong dilution of the serum in the bacterial emulsion, and to dilute this with further amounts of the latter, unless the subsequent dilutions are made quickly. Otherwise, all the agglutinin may be removed by the bacilli in the first dilutions, and those subsequently added be apparently unaltered. The most accurate method is to prepare all the serum dilutions required of double strength, and to add to each an equal volume of bacterial emulsion. On the other hand, when a bacterial emulsion is added to a serum in successive doses, more agglutinin is taken up than when the whole amount is added at once.

Next as regards the phenomena in which an excess of antibody has apparently a reversing action, the best-known examples of which are : (1) the deviation of complement, or Neisser-Wechsberg phenomenon ; (2) the presence of zones of inhibition in which no precipitation occurs in mixtures of serum and precipitin ; and (3) the similar phenomena observable in the agglutinins. All these have been discussed previously and explanations suggested. These explanations have the merit of not involving any phenomena of nature different to those familiar in immunity reactions, but there are objections to all. Thus, Neisser and Wechsberg's explanation of the deviation of complement would have us believe that uncombined complement has a greater affinity for alexin than that which has had its cytophile groups combined with cell receptors—a phenomenon exactly opposite to that which occurs in hæmolysis, where the process can be studied with greater accuracy. We have also seen difficulties in the way of accepting Gay's explanation, the full particulars of which are not yet available. In the same way the inhibiting effects of large doses of precipitating or agglutinating serum may apparently be explained on the hypothesis of the existence of precipitoids and agglutinoids of higher combining affinity than their unaltered antibodies, but it is at least doubtful whether this is entirely satisfactory. It is difficult to believe that the effect of moderate heat is to increase the combining affinity of the agglutinin, whereas greater heat destroys it entirely, and it is altogether different to what is observed in the

case of toxin, which is exactly equal to toxoid in this respect, and Dreyer and Jex-Blake bring forward very strong evidence against this view. Some of the more important of their facts may be summarized thus: If it were true, the more the serum is weakened by heat (and the greater the production of agglutinoid) the greater should be the zone of inhibition, and *vice versa*. This they found not to be the case, for a serum that had had its agglutinating power very largely destroyed by heat might show a very small zone of inhibition, whilst another which had been hardly injured might have a very large one. Further, a serum might show a zone with an emulsion of bacteria in saline solution, but not in broth.

The alternative explanation of all these phenomena is based on facts observed in the mutual precipitation of colloids, for here again exactly similar zones of inhibition are seen. For example, as Neisser and Friedemann have shown, a suspension of particles of mastic in water (made by dropping an alcoholic solution into water) takes on a negative charge, and can be precipitated by positive colloids or by positive ions. Thus, if ferric chloride be added precipitation occurs, and if the dose be increased it gradually becomes more and more rapid and abundant until a certain amount of ferric chloride is present, after which it becomes less and less until no reaction takes place at all. And similar facts have been observed in numerous other cases of interaction of colloids. Their explanation is somewhat as follows: When the two colloids are present in such an amount that their electrical charges mutually annul one another, and they are therefore formed into aggregates which tend to run together, the addition of fresh colloid bearing an electric charge disturbs the conditions, and may, by electrifying the masses already formed, cause mutual repulsion, and, if sufficient amount of the second colloid be added, a re-solution of all the masses. Hence solutions, say, of an electro-negative colloid may be dependent on (a) the presence of mutually repellent molecules or aggregates of molecules in an inert fluid, or (b) on the presence of these molecules in a fluid also containing a large number of molecules of opposite sign, whereas in the intermediate mixtures the conditions for precipitation are present. Here the precipitate is soluble in excess of *both* substances, just as precipitin is soluble in excess either of precipitin or of its antigen.

This theory of the action of agglutinins has been investigated and strongly supported by Biltz, Neisser and Friedemann, Pauli, and others, and Dreyer and Jex-Blake have in particular given

strong evidence in its favour. For example, they find definite zones of inhibition in the precipitation of *B. coli* by orthophosphoric acid, a substance in which the existence of agglutinoids is out of the question. Thus, in a series of tubes each containing 1.5 c.c. of an emulsion of this organism in normal saline solution, agglutination occurred in the tubes to which amounts from 118 centigrammes down to 4 centigrammes were added, and also in those containing from 1.1 milligrammes to 0.01 milligramme, but there was none in which amounts from 4 centigrammes to 1.1 milligrammes were added. Further, there is a close analogy between this phenomenon and the precipitation of gum mastic by ferric chloride, for in each case the extent of the zone of inhibition diminishes as the emulsions of the substance precipitated are made stronger.

The facts at our disposal are not yet sufficient to enable the phenomena of agglutination of bacteria to be fully explained, but the process may take place somewhat as follows: Normal bacteria pass toward the anode when an electric current is passed through the fluid, and are therefore to be regarded as particles carrying a negative charge. The addition of agglutinin removes this charge, and the particles become electrically inert. This is shown by the fact that when placed in an electric current they do not move in either direction. If, however, very large amounts of agglutinin are added, the point of neutrality may be reached and passed, and the bacteria acquire a positive charge, again becoming mutually repellent. Remarkable facts which may have some bearing on this subject have been brought forward by Neisser and Friedemann. Emulsions of mastic are, as we have seen, precipitated by ferric hydroxide, and it has been shown that the addition of a small amount of organic colloid, whether positive or negative or inert like gelatin, protects these particles, so that they are no longer agglutinated by colloids or electrolytes of opposite sign. A phenomenon probably similar is seen when salts which form a colloidal precipitate are mixed in a solution of gelatin, when the mixture remains transparent, the colloidal particles being prevented from undergoing flocculation. Silver chromate, formed by the interaction of potassium chromate and silver nitrate, may be obtained in a transparent and stable form by this process. Particles thus protected are not carried in either direction by an electric current. Neisser and Friedemann have shown that mastic emulsions are "protected" by the addition of a little serum, leech extract, or

extract of typhoid bacilli, and they think that normal bacteria may be compared with these particles surrounded by a defensive envelope which prevents the flocculating action of substances of opposite sign. The action of agglutinin they believe to consist in the removal of this layer, so that then the ions of opposite sign can unite with the bacteria and bring about their agglutination. This explanation would explain clearly the rôle of salts in agglutination, and is supported by Kirstein's observation that typhoid bacilli cultivated in medium free from albuminoid material is clumped by salt without the addition of serum. The theory, however, appears at present not to account for the facts already cited with regard to the transport of normal and charged bacteria in an electric current.

Some remarkable analogies have been brought forward by Landsteiner and Jagic between the process of hæmolysis by simple colloids and by specific antibodies. Thus a solution of colloidal silicic acid acts in a manner closely recalling a specific hæmolysin. It clumps and dissolves the red corpuscles of rabbits, and agglutinates their spermatozoa, but has no action on typhoid bacilli; it thus shows signs of selective action, though not of true specificity. Its action is manifested in extremely small doses; it is rendered inert by heat, and gradually falls off even at the room temperature. Further, phenomena are observable which strongly recall the action of complements and of lecithin in reactivating heated serum or in dissolving sensitized corpuscles, since red corpuscles which have been agglutinated by colloidal silicic acid are dissolved by traces of lecithin or of fresh serum, but not by serum which has been heated to 60° C.; but if the silicic acid is present in excess, no hæmolysis occurs.

A difficulty arises from the fact that emulsions of red corpuscles are agglutinated by both positive and negative colloids (ferric hydroxide, ferrocyanide of copper). Girard-Mangin and Henri have given an explanation of this, which is briefly as follows: When a red corpuscle is suspended in a diffusion of salts, especially of sulphates of calcium and magnesium, takes place from their surface, and these facilitate the precipitation of positive and negative colloids respectively, so that the corpuscles come to be surrounded by a layer of precipitated colloid material. It is this zone of precipitated material which actually determines the agglutination of the corpuscles. Thus these authors consider three substances as taking part in the process: (*a*) the corpuscles;

(b) the salts which gradually diffuse therefrom; and (c) the colloid or agglutinin. They brought forward strong evidence in favour of this theory by showing that agglutination is less powerful when the corpuscles are suspended in normal saline solution (the salts of which inhibit the exosmosis of the salts in the corpuscles) than when they are in isotonic sugar solution (in which exosmosis is unchecked). Further deductions from their theory (for which the original articles must be consulted) were verified experimentally.

The analogies between colloid adsorption and the interactions of toxin and antitoxin have been studied by Biltz, Pauli, and others, and very remarkable facts adduced. For instance, there appear to be phenomena indicating that the action of some antitoxins at least is reversible when a large amount is present. Thus, according to Jacoby, the action of crotin (a phytotoxin of simple nature and closely analogous with the bacterial exotoxins) is increased by the addition of minute amounts of antitoxin, large quantities of which neutralize its activity. Phenomena somewhat similar are seen with the true toxins—for example, Danysz (corroborated by von Dungern and Sachs) has proved that diphtheria antitoxin will neutralize more toxin when added at once than when added in successive portions. Thus if the amount of toxin which is just neutralized by a certain amount of antitoxin be divided into two parts, and added to the antitoxin at an interval of twenty-four hours, the whole may be toxic, although the amounts of toxin and of antitoxin present are exactly the same in the two cases. Both these effects can be explained on Ehrlich's pluralistic conception. Thus Jacobi's results may be explained on the assumption that small amounts of anticrocin combine with the non-poisonous prototoxoid, and that this renders the mixture more toxic, because this prototoxoid would otherwise seize on the receptors of the sensitive cells, to the exclusion of the active toxin. This, however, is very difficult to believe. Taking the action of crocin in producing hæmolysis as a test, it would appear that the neutralization of some of the toxin, even if inert, would render the substance less hæmolytic; this appears to follow from Bordet's proof that red corpuscles can take up much more than their hæmolyzing dose of an antibody. The Danysz effect is also explicable on the theory of the presence of a non-toxic epitoxonoid of no toxicity and of feeble combining affinity. This slowly forms a firm combination with the antitoxin, and renders it useless for neutralizing

more toxin. Explanations are also available on the Arrhenius-Madsen explanation, regarding the combinations as examples of mass reaction.

Regarding reactions such as these as interactions of colloids, we find them paralleled in simple reactions. Thus in some cases the addition to a colloidal solution of a small amount of a second colloid of opposite sign may render the solution more stable, and protect it from precipitation by an excess of the second substance. The partially neutralized aggregates appear to possess more repulsive power than those carrying their full electric charge. Again, the effect of an addition of one colloid to another of opposite sign is often brought about slowly, and the material only gradually attains its permanent form. It is probably this fact that renders solutions of antitoxin so unstable. Interactions take place between the colloids themselves and the electrolytes in the serum, aggregates are formed, and the antitoxic potency falls off—rapidly at first and subsequently more slowly. It is a matter of great difficulty to prepare stable solutions of proteid materials. As we have already pointed out, the amount of colloid necessary to precipitate a constant amount of another of opposite sign is reduced to a minimum if the addition be made at once, and rendered much greater if it is made slowly in small amounts with an interval between each. This is closely analogous with the Danysz effect. And this must also be considered in its bearing on Ehrlich's method of attempting to neutralize a certain dose of toxin by successive addition of small amounts of antitoxin, the method by which the whole of the elaborate pluralistic conception of the structure of toxins has been built up. According to the colloidal theory, this is explicable on the assumption that transitional compounds of toxin and antitoxin of very diverse nature, not necessarily dependent on the proportions of the two substances, are formed. This is practically the view put forward by Bordet from a consideration of the reactions of the antibodies, and especially alexin and anti-alexin, and supported by the workers who have examined the question from the standpoint of colloidal reactions.

The difference between the  $L_0$  and  $L_+$  dose, which is so important a feature in Ehrlich's work, also finds an analogy in the reactions of simple colloidal substances. Thus ferric hydroxide neutralizes arsenious acid (whence its use as an antidote in acute arsenical poisoning), and it was found by Biltz that if one lethal dose of the latter substance was added to a neutral mixture of the



two, this mixture did not regain toxicity. Several lethal doses must first be added, just as it is necessary to add several lethal doses of diphtheria toxin to the  $L_0$  dose.

A difficulty arises from the fact that both toxin and antitoxin pass in the same direction in an electric current. These experiments are naturally difficult to carry out, since the effect of electrolysis must be eliminated. When this is done, according to Field and Teague, both substances travel towards the cathode, and this whether the solution is acid or alkaline. But we must remember that when toxin and antitoxin interact they always do so in a very complex fluid, containing many other substances, both colloids and electrolytes, and until we can determine the electric charge of these substances in a pure form, these experiments can hardly be considered to outweigh the remarkable analogies described above. It is highly probable, judging from analogy with the other antibodies, that free ions are essential to the neutralization of toxin by antitoxin, and the researches of Girard-Mangin and Henri show us how complex the conditions may become.

We have seen that it is important to know whether the compounds of antibody and antigen are dissociable, and that the latter view explains some difficult phenomena seen in immunity. On Ehrlich's theory the combination between the two is a strong one, and once it has been allowed to take place, the two substances are not dissociated into their components, whereas on Arrhenius's and Madsen's theory the combination is an unstable one, and dissociation constantly occurs. The experimental proof in favour of the occurrence of this phenomenon appears on the whole satisfactory. Red corpuscles saturated with immune body mixed with normal corpuscles will part with some of their antibody, so that the latter become sensitized. A mixture of tetanus toxin and antitoxin, which causes no symptoms when injected into the leg of a guinea-pig, causes tetanus when adrenalin has previously been injected locally (explicable on the fact that toxin is absorbed by the nerves and antitoxin by the vessels, which in the second case are constricted), and other examples might be quoted. It appears, however, that this irreversibility only occurs if the two substances are not kept in contact for a sufficiently long time, as was pointed out by Martin and Cherry in their filtration experiments very early in the history of the chemistry of the action of antitoxin. Thus in the latter example, if the tetanus toxin and antitoxin be

kept in contact for two hours, no tetanus is produced, in spite of the local action of the adrenalin. This appears to harmonize best with the colloidal theory. The interactions of colloids of opposite sign and the precipitation of colloids by electrolytes may be reversible if the conditions are changed early, but the compounds formed gradually become more and more stable. But if the continuation were that of two substances of strong chemical affinity we should expect it to be stable from the first, and if it followed the laws of mass reaction it would be reversible, no matter how long it had stood.

There appears to be no crucial experiment by which the truth of these three theories can be tested. The arguments brought forward by the upholders of each involve the study of the exceedingly complicated laws connected with the true relations and completeness of the reactions, and involve advanced mathematical consideration. It is, however, doubtful whether the processes are measurable with the accuracy required in the investigation of these complicated formulæ, and in some cases very slight alteration of the observed results would render the facts explicable on a theory other than that in support of which it was adduced. At the present time the balance of the evidence appears to be decidedly in favour of the colloid hypothesis.

## CHAPTER XIII

### ON IMMUNITY TO BACTERIA

WE are now in a position to discuss the mechanism by which a bacterial infection is combated. It must be pointed out in the first place, and kept in mind throughout, that different microbes are dealt with in different ways. Some, such as many of the protozoan blood parasites, may be tolerated, since the tissues of their host are immune to the action of their toxins; others are removed by phagocytosis, after preparation by opsonins, or perhaps by alexins, amboceptors, or other substances, or possibly without previous treatment; and yet others may be dissolved by the process discussed under the heading of Bacteriolysis. Each of these processes gives rise to a different form of immunity, which we may call—(1) *immunitas non sterilisans*, (2) phagocytic immunity, and (3) bacteriolytic immunity. And the latter two processes (which are the only ones of practical importance) rarely, if ever, exist in a pure state; almost all invading bacteria are, or may be, dealt with by a combination of the two.

We must also notice that the defensive processes will be greatly modified by the nature of the region in which the combat between the invaders and the defensive mechanism goes on. There are three main cases: (1) the circulating blood, (2) the tissues, and (3) regions, such as the peritoneum, combining some of the characters of the other two. We will take them in this order.

In dealing with the processes that take place when bacteria gain access to the circulating blood, our main difficulty at first sight is not to explain the existence of immunity, but the reason why infection ever occurs; for the defensive mechanism seems more than adequate to combat any dose of bacteria which is likely to gain access to the blood under natural conditions. The processes by which the bacteria are removed are phagocytosis and bacteriolysis, or the two combined, and in either case the defence would appear to be more than adequate. Thus, in an experiment for the

determination of the opsonic index in which thick emulsions of bacteria are used the leucocytes are often found absolutely packed with streptococci or tubercle bacilli after a short incubation; if on the average 100 cocci are taken up, calculation will show that the whole volume of blood might phagocyte 2,000,000,000,000 in a few minutes. Tubercle bacilli are perhaps hardly so easily taken up, but it is difficult to see how the comparatively moderate number of these bacteria which gain access to the blood after the rupture of a small caseous gland into a vein can escape immediate phagocytosis: yet we have no reason to believe that this accident often occurs without causing general tuberculosis. Perhaps a more convincing example is given by determinations of the opsonic index in septicæmia, where the bacteria—*e.g.*, streptococci—are known to be circulating in the blood. Here the opsonic index is usually low (0.5 or less), but even then it is sufficient to enable the leucocytes to take up an enormously greater number of bacteria than we can discover in the blood.

Similar phenomena occur in the case of bacteria which are combated largely by means of bacteriolysins. Thus, normal blood serum is markedly bactericidal to typhoid bacilli, yet infection occurs, and many observers have noticed relapses when the bacteriolytic power of the blood is extremely high. In these cases, therefore, the bacteria can gain access to the blood (for typhoid fever is always in part a septicæmia) in spite of barriers which would appear unsurmountable. And we have already had occasion to mention that anthrax may occur in animals the blood serum of which is powerfully bactericidal; the animals, indeed, may be extremely susceptible.

Before attempting to explain the apparent discrepancy, it must be pointed out that, as a matter of fact, septicæmia is a rare disease in proportion to the opportunities for its occurrence, and that the continued presence of bacteria in the blood without a local lesion from which they are constantly discharged is extremely uncommon. Thus in diphtheria, tetanus, the staphylococcoses, etc., in which we should imagine that there is every chance for bacteriæmia to occur, it is practically unknown. It is common in diseases like ulcerative endocarditis, the early stages of typhoid fever, and in pneumonia, in which we have reason to believe that there is a constant shower of organisms discharged from the local lesion direct into the blood. Here, however, we can feel fairly certain that the bacteria which we find in the blood are only there

temporarily, and are rapidly destroyed: the reasons being, firstly, that they are never present in large numbers, as they would be if they lived and multiplied in the circulation, and, secondly, that they do not as a rule form secondary lesions, as we might expect them to do, when deposited in the tissues. We may fairly assume, therefore, that in a disease like typhoid fever bacteria are constantly passing from the lesions into the blood, but are rapidly destroyed, so that the defensive mechanism of the fluid is sufficient to prevent the occurrence of a true septicæmia, in which bacterial growth continues in the blood. The same holds in pneumonia and in ulcerative endocarditis when not accompanied by secondary infective lesions, and probably most other diseases, and does not militate against the view that the blood is a sufficient defence in the majority of the cases in which the infective material gains access to the interior of the vessels. Excluding these and similar examples, septicæmia is a rare disease, and is commoner in the case of the protozoal than in bacterial infections.

As regards the failure of the process of phagocytosis, which must occur when an organism which is combated by phagocytosis does gain a foothold in the blood, we may point out that we have yet but little knowledge of the exact state of the bacteriotropic substances in the living plasma. Wright, it is true, has shown that citrated plasma contains the same amount of opsonin as serum; but the two fluids are not absolutely identical, and the fact that opsonin increases in amount in the successive fractions of serum squeezed out from a clot leads us to believe it probable that it does not exist as such in the plasma; and if, as we have shown is likely, the naturally existent thermolabile opsonin is the same as alexin, it is, on the whole, probable that there is none in the living blood.

Briscoe's experiments reproduce the natural conditions more closely. He injected emulsion of bacteria into the ventricle of a heart after excision and clamping of the auriculo-ventricular groove. After a time some of the blood was removed and examined, and practically no phagocytosis was found to have taken place. But many bacteria were taken up when the same animal's leucocytes and serum and the emulsion of bacteria were incubated outside the body in the ordinary way. Further, in some cases a little clotting occurred in the heart, and in these it was found that the leucocytes which were included within the clot took up many more bacilli than those which remained free.

These results argue strongly in favour of the fact that opsonin does not occur in the circulating plasma as such, and is only set free during the process of coagulation or of phagolysis. This conclusion was corroborated by the fact that opsonized bacteria were taken up freely, showing that the leucocytes were not at fault.

We can get no information on this point from an examination of the blood in these infections, since a small number of non-virulent bacteria will be digested and completely disappear in a few minutes if taken up by a leucocyte; but as a matter of fact, it is very uncommon to find ingested bacteria in the leucocytes of the circulating blood in natural diseases.

It might appear that these explanations go too far, and would lead us to the conclusion that phagocytosis is without value as a defensive process when bacteria reach the blood-stream. This is not the case, and it is probable that the great factor to consider is the spleen. This acts as a sort of filter, and in its pulp the flow of blood is slow, so that there is abundant opportunity for phagocytosis to occur. The same is true, though in a lesser degree, of all regions of the body in which the circulation is tardy.<sup>1</sup> Hence, when bacteria gain access to the blood they will always be found in considerable numbers in this organ, which Kanthack called years ago "the graveyard of the bacteria." This is especially the case in typhoid fever, and the method formerly in use in the diagnosis of the disease, by making cultures from traces of spleen pulp removed by puncture, almost invariably gave positive results. Films or sections of the spleen pulp will often show bacteria ingested by the leucocytes in many diseases, even although none can be detected in the blood. The rôle of the spleen in immunity is probably a complex one, and this may explain the divergent views held as to its importance in this respect. Thus it was found long ago by Tizzoni and Cattani that it was impossible to immunize animals to tetanus after splenectomy; but this was not corroborated by other observers, who found the animals equally immunizable after the results of the operation had completely passed away. But in this connection we must remember that the splenic tissue is very rapidly regenerated, and that in all probability the hæmolymph glands and other structures take on its functions vicariously after its ablation.

<sup>1</sup> Leucocytes which have taken up bacteria whilst in the circulation probably accumulate in the lungs also.

The rôle of the spleen in facilitating phagocytosis is well seen in the spirilloses. Metchnikoff showed many years ago that after death from relapsing fever the cells of this organ are packed with spirilla, such cells being rarely, if ever, seen in the circulating blood. When monkeys are inoculated with blood containing the parasite, a febrile attack occurs, but recovery soon takes place. During the disease free spirilla occur in the blood, but they soon disappear; but if the animals are killed after this, the organisms may be found within the splenic leucocytes. Further, Soudakewitch showed that recovery often failed to occur in animals inoculated after splenectomy, and in this case the spirillum appeared in large and increasing numbers in the blood, and no phagocytosis was observed. Very similar facts have been observed by Levaditi and Manouelian in tick-fever. During the height of the disease the spirillum occurs in the blood, and is exclusively extracellular. After the crisis it is found only in the phagocytes of the spleen and liver (Kupffer's cells).

Besides the spleen, the bone-marrow is also a region where abundant phagocytosis occurs, and the functions of the two tissues, like their structure, are similar. In each case there is a slow flow of blood in the pulp, close proximity of the blood to the tissue cells of peculiar type, and an abundant supply of leucocytes.

The function of these organs in phagocytosis is probably twofold. It provides a region where the blood-stream is comparatively tranquil, so that there is no mechanical obstacle to the process: this is probably comparatively unimportant, since it can and does take place under certain circumstances in the full torrent of the circulation. And, secondly, if, as seems probable, thermolabile opsonin is really complement, and if complement is formed by the leucocytes, it is readily conceivable that it may be present in the leucocytic organs when only present in small amount in the rest of the blood. It may actually be called into existence by the action of the toxin on the spleen cells or leucocytes, and be immediately absorbed by the bacteria, so that it never occurs in appreciable amount in the plasma.

This action will not come into play in the case of the bacteriotropic substances which are true antibodies, for we have every reason to believe that these are present in the plasma as such, and are not set free in the process of clotting; yet even here we have seen some evidence which suggests that they, in common with other true antibodies, are formed in lymphoid tissue or from lymphoid

cells, or, as some recent research seems to prove, from the endothelium.

The liver is also an important region with regard to the deposition of bacteria, and their subsequent destruction by phagocytosis or other means, but in this case the main cells concerned are Kupffer's cells. We must regard these two organs and the lungs as being intercalated in the circulation with the object, amongst others, of arresting bacteria and other foreign particles which gain access to the circulation, and affording a region in which phagocytosis can take place at leisure. It has been shown experimentally that animals will resist a much larger dose of bacteria if the injection be made into the portal vein than if it is thrown into one of the peripheral veins. The importance of these organs is also well shown from a study of the fate of inert particles, such as carbon or carmine, when injected into the circulation or peritoneum. In either case they make their way with great rapidity to the organs, especially the liver and spleen, in which they occur in large numbers within two hours of the injection. They are also found within the bone-marrow, lymph glands, tonsils, and lungs, and, whatever the region in which they occur, are mostly contained within the leucocytes or tissue phagocytes. Here also intracellular absorption may take place, though of course it is much slower than is the case with bacteria, and according to Siebel the leucocytes, with their load of unabsorbable particles, may leave the body via the lung, tonsils, or lymphoid structures of the small intestine.

The lung plays a part of great importance, but one not fully understood, in the process of phagocytosis of bacteria which gain access to the circulation. According to some writers, it is the only internal organ in which phagocytosis by polynuclears can be demonstrated, but this is certainly not correct. It is true, however, that soon after the injection of streptococci (Tchistovitch) or cholera vibrios (Levaditi) into a rabbit, polynuclears containing these organisms may be found in large numbers in the wide capillaries of the lung; this is probably the reason for the sudden fall in the number of the leucocytes in the circulating blood which occurs soon after injection. The reason for this collection in the lungs is very far from clear: the region, remote as it is from lymphoid tissue, would appear an unfavourable battle-ground against the infection; nor are the later stages of the process fully understood.

The question of the importance of the bacteriolysins in hæmic



infections, already alluded to, now requires further mention. It is obvious that the rôle which it plays varies greatly in different diseases. There is, for instance, no evidence for believing that the blood ever develops any substance which is bacteriolytic for the tubercle bacillus. It is true that the researches of Wassermann and his coadjutors show (by means of the method of fixation of complement) that an antibody may occur in tuberculosis or in tuberculous animals, but there is no evidence that this is a bacteriolysin, or, if so, that it ever occurs in amounts sufficient to bring about solution of tubercle bacilli; in fact, it appears probable that tubercle may run its whole course without leading to the production of any recognizable antibody. So, too, with staphylococcic infections. Here it is possible by special methods to produce a serum which has a slight bactericidal effect, but normal human serum or the serum of a patient who has been submitted to a course of antistaphylococcic vaccination is quite powerless in this respect. It is obvious, therefore, that neither the high grade of natural immunity to staphylococci of normal persons nor the increased amount present in active immunity is due to any bactericidal or bacteriolytic substances occurring in the blood. It is quite true that in old specimens of staphylococci pus-free cocci in all stages of degeneration can be found, rendering it quite obvious that a process of extracellular death and destruction of bacteria is at work. This phenomenon is probably to be attributed mainly to the action of the proteolytic enzymes formed by the leucocytes of the pus. These are substances which are too often neglected in consideration of immunity. They are, of course, non-specific, and will act on any dead proteid, and on some living organisms. Their action may be demonstrated by allowing some liquor puris from an old abscess to act (in presence of thymol) on some staphylococci which have been previously boiled to prevent autolysis. Another process in which these degenerated forms of cocci may be produced is by spontaneous autolysis in organisms killed by lack of suitable nourishment, or atrepsy.

In sharp distinction from tubercle and the staphylomycoses, we find such diseases as cholera and typhoid fever, in which bacteriolysins are formed in large amounts, and are readily demonstrable by simple methods. It is in these and a few other diseases that we may expect the rôle of these substances in recovery and immunity to be most marked. We find, however, that their action is most difficult to understand, and that its importance

appears to diminish the more it is investigated. In general terms, there is no doubt as to the fact that the presence of abundant bacteriolytic substances must be regarded as a cause of immunity: thus, animals may be rendered passively immune, *e.g.*, to intraperitoneal injections of typhoid or cholera by antecedent or simultaneous injections of powerful bactericidal serum. But further investigation shows that even in this phenomenon there are certain features which must lead us to regard the process of bacteriolysis as of subordinate importance, or even as harmful. In the first place, the demonstration of the fact that the opsonic action of a powerful antityphoid serum (as determined by the process of dilution until extinction of the property occurs) is proportionate to its bactericidal action, and other facts already noticed, have led us to the belief that immune body or amboceptor can play the part of an opsonin, and, further, that it does so when present in amount so small that its bacteriolytic action is but slight or not apparent. It is, therefore, in the highest degree probable that the passive immunity conferred by the injection of a bacteriolytic serum is due to the opsonizing action of this serum, manifested even when diluted with the juices of the animal into which it is injected.

In the second place, there is this main difference between the destruction of bacteria within the leucocyte after phagocytosis has occurred and the extracellular solution by immune body and alexin: in the former case it is unusual for the endotoxins to be set at liberty, so that as soon as a bacterium has been taken up by a leucocyte, we believe that, as a rule, its capacity for harm has been removed. It undergoes solution within the protoplasm of the leucocyte, which, as appears from the studies of the French school, is peculiarly insusceptible to the action of toxins, is destroyed by the digestive juices, or by other means, and is not, at least in the majority of cases, set free to injure the tissue cells. It is otherwise with the solution of bacteria which takes place from the action of bacteriolytic antibodies, for here the endotoxins are set free, so that the presence of these antibodies, though undoubtedly developed as a part of the defensive mechanism, may be a source of added danger to the animal. For example, if an intraperitoneal injection of a large dose of cholera vibrios be made into the peritoneal cavities of two animals, and in one some anticholera serum be added, it is often found that this animal dies very rapidly with symptoms of acute intoxication, whilst the other survives longer

and dies of septicæmia. If dead vibrios are used the addition of serum may bring about death, whilst an animal injected with the organisms alone may recover with scarcely a symptom of intoxication. Similar phenomena, though less definite, often follow the use of antistreptococcic serum for patients suffering from streptococcic disease: the injection may cause a rapid rise of the temperature and exacerbation of the symptoms. Other explanations are possible, but it is highly probable that this is due to the solution of some of the cocci and liberation of their endotoxin. It is possible that in some cases this might be a source of danger, and that this liberated toxin might be sufficient to kill the patient; but there is no evidence that this actually occurs, and, as a rule, the phenomena mentioned may be taken as proof that the serum is a suitable one, and that its use should be continued.

We must, however, be careful in arguing from the phenomena occurring in hypervaccinated animals to those suffering from a natural infection. There can be no analogy between the train of events when a massive dose of pathogenic bacteria is suddenly injected into the circulation or peritoneal cavity of an animal in which there is already an abundance of bacteriolytic substances, and those which follow a natural infection. Considering the latter case, and excluding all considerations of phagocytic action, we may trace the sequence of events in the case of a disease such as typhoid fever somewhat as follows: In the first place, since normal human serum is bactericidal to typhoid bacilli, we must regard the normal resistance against the disease as being due, in part at least, to the presence of immune body in the serum, though we have no means of knowing whether its action is mainly opsonic or mainly bacteriolytic when occurring in the plasma. When infection occurs, the amount of this substance must diminish, since it will combine with the typhoid bacilli to which it gains access, and may, indeed, be sufficient to kill them all, and thus prevent the development of the disease. Where, however, the organisms gain access in sufficient numbers, so that the amount of immune body or of alexin is insufficient to prevent the further growth of some of them, those that escape will continue to grow, and the disease will gradually increase in severity. At the same time, the dissolved products of the organisms which succumb will reach the seats of antibody production and stimulate a fresh production of immune body. We have seen reason to believe that this production takes place mainly in the lymphoid tissue and

other regions rich in lymphocytes, and we may probably corroborate this fact with the hyperplasia of the abdominal lymph glands, Peyer's patches, and spleen which occurs in the disease, regarding them as conservative, defensive phenomena, rather than the pathological effects of the bacillus.

After a latent period or negative phase of a few days (the duration being dependent on the severity of the infection), the antibodies begin to make their appearance. At first put out only in small quantities, they will be absorbed at once by the bacilli present, and will not be demonstrable in the serum or plasma in a free state. A race now ensues. On the one hand, the typhoid bacilli multiply as rapidly as their environment will allow; on the other, the immune body and other defensive antibodies are being gradually elaborated more and more rapidly. In a very severe infection this latter process may perhaps make default altogether, and the patient succumb during the negative phase. This has not been demonstrated in the case of the bacteriolytic substances, but is well known in the case of the agglutinins. In most cases, however, the increase of bacteria and of antibodies takes place at the same time. After a time bacteriolysis occurs, but in small amount, since but little immune body makes its appearance, and that which is formed at first is probably utilized as opsonin. Hence when solution of the bacteria and liberation of the toxins does occur, it does so at first only to a small extent, and we may regard it probable that the minute amount of endotoxin set free does not add appreciably to the symptoms of intoxication already present. Further, we have now the conditions under which immunity to these toxins may be expected to develop: the setting free of small but gradually increasing doses of endotoxin—conditions, in fact, closely similar to those obtaining in Macfadyen's experiments. Probably, therefore, by the time that much bacteriolysis occurs the patient's tissues are more or less immunized. It is, however, not unlikely that the rapid oscillations of temperature characteristic of the latter stages of typhoid fever may be due in part to this liberation of endotoxin.

Some researches by Bail would appear to indicate that there is a sort of automatic mechanism for the prevention of bacteriolysis in the tissues. He found that no bacteriolysis took place when a powerful serum was mixed with emulsions of cells from the liver, spleen, etc., as well as with bacteria. The explanation is doubtless that the complement is absorbed by these cells, as has been pointed

out by Hoke, Muir, and others. Now, if this process goes on in the living body, it can only indicate that complement (and thermolabile opsonin) can never occur in a free state in the organs, and hence that bacteriolysis does not occur in these regions. It must be pointed out, however, that amboceptor (or thermostable opsonin) is not absorbed in this way, so that there would be but little interference with phagocytosis. It is quite possible that this absorption of complement does take place in order to avoid the liberation of endotoxin consequent on bacteriolysis.

The mobilization of the patient's defences is usually manifested by an increase in the number of the leucocytes in the circulating blood. This subserves two functions: there is a greater number of leucocytes to act as phagocytes and there is an increase in the possible number of cells which may produce alexin or complement. Hence we find leucocytosis present almost invariably in cases in which bacteria gain access to the blood; the exceptions being, firstly, very severe infections in which the defensive reaction fails, and, secondly, a group of diseases of which typhoid fever is the best example. The meaning of the first exception is tolerably clear, that of the second not at all apparent.

The mechanism by which this increased output of leucocytes is produced is *chemotaxis*. The bacteria in the blood-stream produce a toxin which, in comparatively mild infections or in severe infections in an animal possessed of strong natural immunity, attacks the leucocytes. These cells will, therefore, tend to leave the region in which they are formed and in which they are lying dormant and make their way into the blood, the amount of toxin being greater in the latter than the former situation. At the same time, as Muir has so conclusively shown, there is an increase in the functional activity of the bone-marrow, which manifests itself in an increase of the leucocyte (and especially polynuclear leucocyte) producing cells. A double process goes on, leucocytes being formed more rapidly and attracted from the bone-marrow as soon as they are produced. That this is due to chemotaxis is shown by the fact that it follows the injection of bacterial toxins and other products into the blood-stream, if not too virulent or in too large amount.

When either of these conditions occurs we see the opposite result—a leucopænia, or at least an absence of leucocytosis, and this is always an extremely bad omen when it occurs in those infectious processes where leucocytosis ordinarily takes place.

Thus in pneumonia the number of leucocytes per cubic millimetre has a most important prognostic value, and in almost all cases it will be found that a patient with a high leucocytosis will recover, even if the attack is a severe one and the clinical signs unfavourable. This leucopænia may be attributed either to negative chemotaxis, or to the production of paralysis or death of the leucocytes, or to inhibition of the functions of the bone-marrow, or to a combination of these causes. In any case, it argues a high grade of virulence on the part of the bacteria, which, by lessening the action of the main natural protective forces, increases the chance of a lethal issue to the disease. Let us, therefore, discuss some of the main facts (many of which have been referred to before) concerning the nature of the mechanism of an increase in virulence.

For an organism to be virulent it must produce a toxin which has a profound action on the cells (and, it may be added, on the important cells) of the animal in question. This, of course, is obvious, and without it the organism could only produce pathogenic effects by such means as deprivation of oxygen or food-stuffs from the host or the production of bacterial emboli—results which are of no practical importance. Without an active toxin the bacteria would either lie latent, as occurs in typhoid fever, gonorrhœa and tubercle, or circulate in the blood as a harmless parasite, as is the case with many protozoa in the lower animals.

As regards the special actions of toxins which render the bacteria especially virulent, we may distinguish two—(1) a leucocytic or leucolytic action, and (2) negative chemotaxis. The former is a very common function of highly virulent bacteria; its action is best seen in the production of pus and necrosis in local lesions, but its presence may be inferred from the degenerative changes frequently present in leucocytes in general infections. And these leucocytic substances need not be true toxins, for there is reason to believe that the non-specific enzymes and other substances which the bacteria produce have this action, and that the death and destruction of leucocytes which is so marked a feature of abscess production may be due in part to their action.

We have already referred to the question of negative chemotaxis. It probably actually occurs, but the proof is hardly convincing, and the subject needs investigation by modern methods *in vitro*. There is no doubt, however, that in virulent infections the regions

containing the bacteria are often singularly free from leucocytes, be the explanation what it may.

The first necessity for virulence, therefore, is the possession of an active toxin, which, either by lowering the general vitality, or by repelling, paralyzing, injuring, or killing the leucocytes, diminishes the amount of phagocytosis which occurs.

A second defence against phagocytosis is the production of a defensive envelope. This is well seen in the case of virulent anthrax bacilli, which form thick gelatinous envelopes in the animal body. Such capsulated forms are only taken up with difficulty by the leucocytes, and the greater the power of capsule formation the greater the virulence of the culture. Similar phenomena have been seen in the case of the streptococci, tubercle bacilli, and other organisms. The capsule of the pneumococcus, which is only developed in the animal body or in culture fluids approximating thereto, is probably a case in point.

Capsule formation is also probably a defence against bacteriolysins. Thus, according to Eisenberg, virulent typhoid or coli bacilli stain blue by Giemsa's method, and show a pink edge. Organisms which have undergone this modification are susceptible to phagocytosis, but show great resistance to bacteriolysis. And it is probable that the capsule of the anthrax bacillus is intended largely as a defence against bacteriolytic substances. The bacilli can be trained to produce it by cultivation in the serum of the rat, which dissolves the unaltered bacilli in large numbers.

A third and more subtle method in which a bacterium can increase its virulence is by loss of the receptors (which it possesses in the avirulent state), which have the power of anchoring the defensive substances of the blood. Thus the virulent pneumococci which (as shown by Rosenau) are not ingested by leucocytes in presence of serum escape, as a result of the fact that they do not absorb opsonin. Typhoid bacilli which have been cultivated in immune serum lose their power of combining with amboceptor, and also with agglutinin, and it may be taken as a general rule that the more virulent a culture the less its reaction to its agglutinin, and the less it absorbs that substance. Thus typhoid bacilli, when isolated from the body, are usually refractory to agglutination, and gain the property only after several generations of culture on artificial media.

Hence, therefore, we may picture the process which goes on when a dose of pathogenic bacteria gains access to the blood of a

susceptible animal somewhat as follows : The bacteria form a toxin which attracts the leucocytes from the bone-marrow into the blood, and which also stimulates their production in larger numbers. It also attracts the leucocytes into the neighbourhood of the bacteria, and this process is more marked in regions, such as the spleen and bone-marrow, in which the flow is slow. As a result some leucocytes are stimulated to produce complement, or, on the other theory, are killed, and their complement set free. In either case the bacteria are prepared for phagocytosis. This process may now take place, the bacteria be destroyed, and the infection come to an end. This is a type of a mild infection.

Or it may happen that the toxin is so powerful that the leucocytes are repelled, or, if attracted, immediately killed. If this occurs the sole resource of the patient is the bacteriolytic property of the blood, which will then come into action—provided, of course, that the bacterium is one to which amboceptor occurs in the normal blood—because alexin is set free in the solution of the leucocytes. Experiment, however, leads us to believe that this is a slower and less important process than phagocytosis, and that if the latter is in abeyance the outlook for the patient is bad in the extreme. If both processes fail the infection must be rapidly fatal.

In an infection in which the defensive and offensive mechanisms are nicely balanced, and an illness of some duration and severity occurs, the processes will be much more complex. The early stages will occur as in a mild infection, and the bacteria will be surrounded by the leucocytes, and perhaps even ingested. In this case it may happen that after ingestion solution will take place, and an endotoxin be set free which will kill the phagocyte, and perhaps also those in the immediate neighbourhood, and in this way some of the bacteria may be shielded from further phagocytosis for a time. This and similar processes give the bacteria time in which to undergo their defensive modifications, which they do in virtue of the "survival of the fittest"—*i.e.*, those which are best adapted to their environment in the host. Now it is clear that in any culture of bacteria the different individuals have marked differences with regard to their power of absorbing antibodies. This is obvious when we consider that otherwise the addition of gradually increasing amounts of an agglutinating serum would cause no effect until a certain concentration was reached, when *all* the bacteria would clump. As it is, those organisms with the greatest affinity take up the agglutinin in



largest quantity, and when the number of molecules reaches a certain amount they clump, whilst those with less affinity have not yet absorbed sufficient. Probably exactly similar phenomena occur in the opsonization of bacteria. Some organisms are avid for opsonin; others take it up with difficulty, and a large concentration of the substance is necessary before they are prepared for phagocytosis. This probably explains the fact that the number of bacteria taken up by the leucocytes does not increase *pari passu* with the amount of opsonin present.

The bacteria which remain immune to the defensive mechanisms will be enabled to live, and in their descendants the conditions for natural selection will occur—variations and an adverse environment. The weaker forms—*i.e.*, those which, by the absence of a defensive layer or the presence of numerous receptors on which the opsonins or bacteriolysins of the host can seize, or those which do not elaborate a powerful toxin—will be destroyed, and the more virulent forms will survive, so that a gradual selection of the latter will ensue, and the invaders rapidly increase in virulence. And it must not be forgotten that bacteria multiply with great rapidity, division sometimes occurring in a quarter of an hour, so that nearly a hundred generations are passed through in a day, and abundant opportunity for evolutionary selection occurs. From what we know of the virulence of typhoid bacilli and of pneumococci when causing disease and when living parasitically without the body, there is reason to believe that this increase of virulence always takes place in infections.

Here, then, the standing forces of the body are insufficient to deal with the invader, and the latter increases in virulence and numbers during the early stages of the struggle. This process will continue until the latent reserve forces are mobilized and fresh defensive substances brought into action. These are (1) antitoxins, whether to exotoxins or endotoxins, or the toxins may be dealt with in one or other of the methods discussed under Antitoxic Immunity; but in any case it is probable that the development of some degree of toxic immunity, especially, perhaps, of the leucocytes, must precede the destruction of the bacteria. (2) Increased amounts of alexin-opsonin having a specific action on the organism in question. We have already noted some of the gaps in our knowledge of this subject, and shall, perhaps, again have occasion to refer to the difficulties in explaining its action, but there can be little doubt of its importance in

generalized infections. We notice, for example, that in pneumonia it remains at a low level during the attack, and increases rather suddenly at the time of the crisis; the pneumococci disappear from the blood at this period, though they remain living in the lung until much later. In cases which recover by lysis, on the other hand, there is a *gradual* increase in the opsonic index, and in acutely fatal pneumococcal septicæmia the index falls gradually and continuously. When dealing with *generalized* infections the opsonic index (as far as our researches have gone at present) may be taken as a very rough guide to the degree of immunity and chance of recovery. This does not apply in local infections, but here we are probably justified in believing that the higher the index, the less the chance of generalization. It is worthy of notice that the disease—cerebro-spinal meningitis—in which the highest indices (sometimes as high as ten) are found is one in which bacteriæmia very rarely occurs, though by analogy with other similar diseases we should rather expect it to do so. As regards the source of this increased amount of alexin-opsonin, there is nothing to be added to what has been already stated.

(3) In some cases recovery may be brought about by the production of thermostable opsonins, whether amboceptor (as appears most probable) or agglutinin. This is rarely, if ever, the case in the pneumococcic diseases, but probably occurs constantly in the maladies, like typhoid fever and cholera, in which agglutinins and bacteriolysins are formed in large amount. We may, indeed, classify diseases roughly into two main groups in this respect: (1) those in which the acquired immunity is due (*inter alia*) to the presence of thermolabile opsonins: in these we may expect the degree of the immunity to be but slight and its duration short; and (2) those in which it is due to true antibodies: here we may expect the opposite conditions to hold. Pneumonia and typhoid fever may be taken as examples. We do not know accurately the duration of the immunity to the latter disease, but the antibodies on which we believe it to depend may be traced for long periods, whereas the blood returns to its normal state very shortly after recovery from a pneumococcic infection. And experience with typhoid vaccine leads us to suppose that the immunity to that disease lasts a year or more. The occurrence of relapses may seem to argue that the protection is in reality very evanescent, but other interpretations are possible.

The processes which occur in *local lesions* are similar in

nature, but modified by their occurrence in the tissues and not in a fluid medium, and they tend to deviate more from test-tube conditions than do the blood infections.

At the commencement of the infection the conditions are quite comparable to those of a hæmic infection. Leucocytes are soon attracted into the area, and, if the bacteria are not too virulent, may remove them entirely, the infection being thus cut short at its very commencement. Probably myriads of slight wounds are thus dealt with. But when the bacteria resist phagocytosis, and form virulent toxins, a new set of factors are brought into existence. These have been glanced at previously, and will now require further discussion.

The dilatation of the vessels of the infected region and the consequent acceleration of the blood-flow is, as has been pointed out, altogether a conservative influence, having for its object the removal of toxins and their dilution in the general mass of the blood. But in all cases except the most trivial the local reaction extends further, and the more extensive changes tend, partly at least, to favour the spread of the infection by shielding the bacteria from the full action of the blood. In other words, the middle stages in the evolution of an inflammatory lesion indicates a victory for the bacterium in so far that it has succeeded in altering the tissues to such an extent as to shield itself from the action of the protective forces. At a still later stage (in lesions going on to recovery) the tissues and juices gain the victory, and there is an alteration in the nature of an increase in the local, and usually of the general, immunity.

Let us trace what we know of this process in the case of a *small* staphylococcic lesion. The early stages consist of the usual inflammatory reaction. The increased access of blood, increased number of leucocytes, and probably increased amount of opsonin, present in the tissues may suffice to cut short the process, the whole of the bacteria being removed by phagocytosis. In this case the only clinical signs will be those of acute inflammation, resulting in rapid recovery.

If, however, the bacteria are present in too great numbers, or are too violent, or if the immunity (local or general) is deficient, the toxins formed will kill the tissues in the neighbourhood, and the bacteria will then lie in a smaller or larger mass of necrotic tissue, which is surrounded by an inflammatory zone. The tide has now turned very definitely in favour of the bacteria, for four

reasons: (1) There is a mechanical obstacle to the access of leucocytes to the bacteria, which make their way through the slough with some difficulty. In ordinary healthy tissues, permeated by capillaries, the leucocytes have never very far to go to reach a given area; but when death of these tissues has occurred, the leucocytes have to crawl in from the still patent capillaries of the periphery, a distance, maybe, of several millimetres. (2) The bacteria in the centre of the lesion have time to elaborate a powerful toxin, which is not diluted by the blood or lymph, since the vessels are all thrombosed, and can only escape by diffusion. As a result, there is a central zone where the toxin is present in so high a concentration that it may either repel the leucocytes by negative chemotaxis or kill them outright. The latter process certainly takes place, as the large number of dead bacteria present in pus sufficiently proves. (3) There is an insufficient supply of opsonin, and perhaps of other defensive substances also, in the fluids at the centre of the lesion. This is brought about partly by removal of these substances by the bacteria of the region. In the case under consideration the staphylococcus opsonin is absorbed by the cocci which, in the absence of the leucocytes, it is powerless to injure. Another possible factor in the removal of these substances is the action of proteolytic enzymes, the defensive materials being digested and rendered inert before they reach the bacteria. Lastly, there is reason to think that opsonins make their way with difficulty through the inflamed tissues. Thus Bulloch found that the serum or liquor puris which was collected from an abscess immediately after it had been cleansed was almost devoid of opsonic power, and this is the case with the fluid portion of pus in general. In some cases I have been able to demonstrate the existence of an antiopsonin, perhaps consisting of cast-off receptors of the bacteria. (4) There is an increase in the virulence of the bacteria, due to conditions already discussed.

The absence of opsonin from the fluid at the centre of the lesion suggests several considerations of some importance. We see, for instance, that it is not sufficient in the cure of a local lesion for there to be an abundant supply of opsonin in the blood: two other factors are required—a permeable lesion and a region suited for the activity of leucocytes. This is very well seen in the case of tubercle, where the opsonic index may be greatly raised (to 1·8 or more), and the lesion show no indication of recovery. There is, as a rule, little or no tendency of the disease to spread or

become generalized with a high opsonic index, yet even this may occur. We may trace a certain degree of parallelism between the ease of access of opsonin to all parts of the local lesion and the likelihood of a cure following an elevation of the opsonic index. For example, there is, according to Tunncliffe, a definite relation between the rise of the opsonic index and the disappearance of the membrane in diphtheria, the latter clearing rapidly when the index rises above normal. Here the conditions are these: there is a membrane, a few millimetres thick, composed of dead material, in which the specific bacilli are elaborating their toxins; below this there is a zone of acute inflammation, hyperæmic and infiltrated with phagocytes. Now it is easier for the toxins to diffuse outward and be washed away by the secretions of the mouth than to pass inwards, and probably but a small fraction actually formed reaches the blood-stream. On the other hand, it is comparatively easy for the protective substances in the serum to pass outwards, the conditions being quite different from those in a closed abscess, where opsonins, etc., can only reach the centre of the lesion by diffusion, and not by actual transudation. Here there is a constant stream of lymph from the pervious vessels to the free surface. Thus the low concentration of the toxins renders it easy for the leucocytes to gain access to the bacilli, and the latter have abundant opportunities of becoming opsonized; hence the conditions for successful phagocytosis are produced.

A similar train of phenomena follows the free drainage of an acute abscess. The toxins escape outwards, and are no longer forced into the tissues, and at the same time there is a flow of protective lymph into the abscess cavity, and consequent sensitization of the bacteria, and the removal of the toxins allows the phagocytes to act. If, however, the wall of the abscess is very thick and impermeable, it may be, as in Bulloch's experiment, that the lymph which exudes is deprived of its opsonin and other protective substances during filtration, and the conditions are then less favourable.

Now consider a *large* staphylococcic lesion completely embedded in the tissues. There is a large core of dead material, in which the cocci constantly produce toxins, and in which they are completely shielded both from fresh lymph or plasma and from leucocytes. Here we should expect the lesion to be progressive, no matter how high the opsonic index, and this is usually the case. Hence, other things being equal, it is in the smaller lesions that we may expect

benefit from vaccine injections which raise the index, and more especially in cases in which there is a free drain for the toxins—ulcers, open abscesses, sinuses, etc., or in those in which there is no dead material (slough or caseous matter), and the blood is brought into close contact with the bacteria. Small isolated tubercles in the iris often yield rapidly to tuberculin injections, whereas large caseous glands are most refractory.<sup>1</sup> Chronicity is also an important factor: a long-standing thick-walled abscess is less amenable to treatment than a small freshly formed boil.

In order to aid this flow of plasma or lymph laden with protective substances through the lesion, Wright has suggested the exhibition of substances such as citric acid or leech extract, which have the power of diminishing the coagulability of the blood. In certain cases the beneficial effects of these remedies may be most striking. And the local use of hot fomentations, etc., which dilate the vessels, has a similar effect; in addition to which they probably aid phagocytosis by raising the temperature of the part.

Next as regards the other pre-existing defensive substances of the plasma—the bacteriolysins. In the case of the staphylococci there is no reason to think that they are of any importance, since most authorities hold that there is no proof that serum has any bactericidal action on these organisms under any circumstances.<sup>2</sup> And when the blood does normally contain the amboceptor-complement apparatus, the establishment of the lesion sufficiently demonstrates the insufficiency of this apparatus for defence.

At a later stage, supposing the pre-existing defences—opsonin and leucocytes, etc.—fail to bring about cure, a second series of factors come into action. These are the antibodies, the chief being antitoxin, amboceptor, and thermostable opsonin, the latter being possibly either agglutinin or amboceptor, as we have seen already. These usually take about a week to be produced, and may be looked upon as the second line of defence.

As regards their place of production, this may be remote or local. The sites of general production of the antibodies have

<sup>1</sup> In saying this I do not wish to imply that the cure in these cases, and after the use of vaccines in general, is brought about *solely* by an increase in the opsonins of the blood.

<sup>2</sup> I must point out, however, that in old staphylococcic abscesses it is common to find cocci which have lost their power of retaining Gram's stain, an invariable proof (where applicable) of the early stages of bacteriolysis. This is probably due to the action of the peptic enzyme formed by the leucocytes.

been discussed already, and we have seen reason to believe that the chief regions in which it takes place are the lymphoid organs, the lymph glands, bone-marrow, and spleen especially. These organs appear to have as one of their functions the elaboration of defensive antibodies as an internal secretion in response to toxins and other bacterial products (free receptors, etc.) circulating in the blood. We have also seen that the phagocytes, and especially the polynuclear leucocytes, may act as sources of these bodies, but that the evidence is less convincing than in the case of the lymphoid organs.<sup>1</sup>

The local production of antibodies is probably more important, since it takes place at the spot at which these substances are required. As an example we may take Römer's demonstration of antitoxin in the conjunctiva as a result of installations of abrin, when no antitoxin had yet appeared in the general circulation. The source of these antibodies, as first suggested by Whitfield, is probably the lymphocytes, which form so important a factor in chronic inflammatory lesions, and which occur after a few days in the periphery of acute lesions. These cells do not act as phagocytes *in vivo*, though they may do so under experimental conditions, and on any other hypothesis their presence in inflammatory lesions is entirely inexplicable. We may feel certain, however, that they are attracted there for some good purpose, and it is in the highest degree probable that this purpose is the elaboration of protective antibodies. It seems fairly certain that the source of the hæmic antibodies is the lymphocyte cells of the adenoid tissues, and the cells of a chronic inflammatory lesion are exactly similar. And in many cases if we examine the region in which inflammation has taken place some time previously, we shall find that the cells which were attracted thither have become organized into definite adenoid tissue.

It seems probable, too, that these small round cells are the basis of the local acquired immunity, concerning which so little is definitely known. After the bacteria have been destroyed the polynuclear leucocytes remove the débris of dead tissues, etc., and then retire, but the lymphocytes persist in the region for long periods. During this time they are perhaps continuously elaborating small amounts of antibodies, and are certainly on the spot and ready for action should a fresh infection occur. It is probable,

<sup>1</sup> More recent researches tend to point to the endothelial cells as the more probable source of origin of the antibodies.

too, that they may have become so altered in virtue of having once been stimulated to produce antibodies that they can do so more quickly and easily than normal cells. Thus Cole has shown that an animal which has once been inoculated—*e.g.*, with typhoid bacilli—will produce on a second injection a much greater output of antibodies than will a normal animal, and that this increased defensive reaction persists for months, long after the animal has apparently become normal. This observation is probably of the highest importance, both as regards general and local acquired immunity.

Thus in the case of a local lesion we may distinguish two stages: (1) That in which the natural resources of the body alone come into action, and in which the main mechanism for combating the bacteria is phagocytosis, the main cell the polynuclear leucocytes, and the main defensive substance the thermolabile opsonin. In this stage there is in general a decline of the local immunity due to the action of toxins on the tissues, and the only rise of the general immunity, if present at all, is due to an increase of this opsonin. (2) The second stage is that of the antibodies. The factors taking part in the first stage persist, but there is a new defensive cell, the lymphocyte, and new defensive substances, the antibodies. Here the immunity, both local and general, tends to be raised. We may also distinguish a third stage, in which the polynuclears have retired and the lymphocytes remain—a stage in which the natural immunity of the part is reinforced by the actual or potential opsonins due to the lymphoid cells, and in which these are better equipped (in virtue of their previous training) to produce a large amount of antibody in response to a slight stimulus.

Lastly, we must study briefly the defensive reactions of the peritoneum, a region which has been subjected to a full investigation and which resembles the tissues in some points and the blood in others. In all probability the process of absorption from the other serous sacs is, in general, similar. We have already glanced at the subject, and in what follows much use has been made of the admirable researches of Buxton and Torrey. The case of an intraperitoneal injection of a pathogenic organism of moderate virulence into an animal possessed of a certain amount of natural immunity will be considered—*e.g.*, of a laboratory culture of *B. typhosus* into the peritoneal cavity of the rabbit.

Under these circumstances, one or other of two trains of events



may take place. In the first the immunity appears to depend mainly on bacteriolysis, in the second on phagocytosis.

In the former case an enormous number of bacilli are killed in the space of a few minutes, very few being recoverable from the peritoneum by washings with normal saline solution, and when this takes place it is found that few or no bacilli make their way into the blood or organs. In such cases the typhoid bacilli which are recovered show marked signs of degeneration, similar, though less marked, to those seen in cholera vibrios in the classical Pfeiffer's reaction; the staining material becomes collected into the centre of the sheath of the bacillus, which subsequently breaks down into minute granules. This process is usually entirely extracellular, but after a time some of the altered bacilli may be taken up by the phagocytes. It would appear at first sight, therefore, that in this case the complement-amboceptor mechanism is present in the normal peritoneal fluid, ready to bring about immediate bacteriolysis. This, however, is by no means certain, and it is highly probable that the complement does not exist in this fluid, and that its appearance follows the injection, and is due in some way to the leucopænia which has been already noted. The nature of this leucopænia has been much discussed, and is of some importance in regard to this question of the nature of the complement. Metchnikoff and the French school generally maintain that the diminution is due to the actual destruction of the leucocytes—phagolysis—and that during this destruction or solution of these cells alexin or complement, or, as Metchnikoff calls it, microcytase, is set free. As a matter of fact, there is no evidence that this phagolysis does occur, and more recent researches appear to point to another solution of the leucopænia. It is true that the fluid withdrawn from the peritoneum by means of a pipette is markedly deficient in cells, but those that are present do not show the marked degenerative changes we should expect if they were undergoing rapid solution. And in any case, it is difficult to believe that an injection of substances such as normal saline, which we know preserves the leucocytes in a lively condition for many hours, should have such a profound destructive effect on them in the peritoneum. Normal saline, it may be pointed out, is capable of producing a most marked leucopænia when injected into the peritoneal cavity.

The most probable explanation of the phenomenon is that first suggested by Pierallini, and corroborated by Buxton and Torrey.

According to them, there is a deposition of masses of fibrin on the omentum, and in and upon these masses there are numerous polynuclear leucocytes.

The formation of fibrin, it need hardly be pointed out, is universally regarded as being due to the liberation of fibrin ferment by the leucocytes. It is not yet settled beyond controversy whether this is to be regarded as a process of secretion, a vital phenomenon, or a process occurring only during the solution—phagolysis—of the leucocytes. The point is of no importance: the remarkable feature is that, according to the opinions we have considered as most probably correct, complement is set free at the same time as fibrin ferment. The deposition of fibrin on the surface of the omentum may be taken as sufficient proof of the liberation of this substance, so that the rapid destruction of the bacteria which occurs in the peritoneum in some cases cannot be regarded as constituting definite proof that it occurs preformed in that situation.

In some cases, therefore, bacteria injected into the peritoneum are killed rapidly, almost instantaneously, by a process resembling bacteriolysis, and doubtless due to amboceptor existing in the peritoneal fluid as such at the time of the injection, together with complement which is probably set free from the leucocytes as a result of the presence of the foreign body, but which may possibly also be present in the normal state. As an example of this process, Buxton and Torrey quote the case of a rabbit which received an intraperitoneal injection of about 4,000,000,000 typhoid bacilli, and was then *immediately* killed, the peritoneum washed out with normal saline solution and plated out. The fluid contained only about 1,000 bacilli, and there were none in the blood or internal organs. In a case which was allowed to survive for two hours no bacilli were found in any part of the body. It is most remarkable that, with all this sudden, almost explosive, solution of bacilli there were no symptoms indicative of the liberation of endotoxins, the temperature remaining constant. The culture was one of very moderate virulence.

In other cases the whole process differs, and the defence of the body appears to be entrusted to the phagocytes rather than to the bacteriolytic substances, and in these a most interesting train of phenomena occurs. Some destruction of the bacilli may occur in the peritoneum, showing that the bacteriolytic properties do not fail entirely, but comparatively few organisms are destroyed by

this process ; instead, large numbers of bacilli make their way into the circulation by a route not fully known. These rapidly decrease in number, the diminution being quite noticeable within half an hour, and in six hours or less the blood may be entirely sterile. There is, further, a remarkable amount of deposition of the bacilli in the organs, especially the liver, spleen, lung, and bone-marrow ; and in these situations the numbers of organisms decrease for about two or three hours, and then show a very definite rise, which lasts for two or three hours more, and the organisms may attain numbers approximating to those present in these organs immediately after the injection. The numbers then gradually decrease for the next two days or so. Here we may see in a very clear manner an example of the mobilization of the defensive forces discussed previously. The diminution which takes place immediately after the access of the bacilli to the organs is doubtless due to the bacteriolytic substances, either present in the blood as such, or immediately available after phagolysis or secretion of alexin. After a time these substances are exhausted, and it is only after some hours that a fresh supply is elaborated, and destruction of bacilli continues. Further, the process that now takes place is mainly phagocytosis, whereas the earlier defensive process was bacteriolysis. This sequence of events is probably to be interpreted as follows : As soon as infection takes place the small amount of immune body naturally present in the blood combines with the bacilli, and, since some complement is present, bacteriolysis occurs. In this way all the immune body is removed in combination with the bacilli, and all the complement is also absorbed, since the conditions for the Bordet-Gengou phenomenon are present, and complement not actually required for solution of bacilli will be taken up, as well as that which is actually used in the process. Hence *all* the defensive substances are removed, and the bacilli can flourish unchecked for a time. Soon, however, more complement is produced. This has little or no power of producing bacteriolysis in the absence of amboceptor, but it can, and does, act as opsonin, and abundant phagocytosis occurs. In most diseases this stage would be marked by leucocytosis, but in typhoid fever this does not occur. But we shall shortly see that a polynuclear reaction does take place in the peritoneum, if not in the other regions.

In the series of researches we are discussing the development of the last line of research—the true antibodies—was not studied.

These only come into action later. In the case of a mild infection the whole process of cure may occur without their appearance, and they are only of importance in so far as they are the cause of the subsequent immunity.

Let us now turn to the sequence of events in the peritoneum in these animals in which explosive bacteriolysis does not occur, or only to a small extent, in that region. Here some phagocytosis is found to take place in the cells lying free in the peritoneal fluid, (the changes in which have been noted previously), but the greater part of the process—and this is significant in view of the theories which we have adopted with regard to the liberation of complement-opsonin during the process of coagulation—takes place in the masses of fibrin found on the omentum. At first the bacilli lie free in this deposit, often lying more or less parallel; but in a short time vast numbers are taken up by the macrophages, and very few free organisms may be seen in an hour or two after the injection. Then one of two series of phenomena may occur. The animal may recover; and in this case the bacilli which are contained in the macrophages become pale and granular, and soon disappear altogether, and the fibrin becomes infiltrated and eroded by polynuclear leucocytes. Or the animal may die; and in this case the bacilli, both those which have been ingested and those which have not, commence to grow rapidly, and the organisms, after diminishing in numbers for a few hours, proliferate so quickly that the animal dies in some twenty-four hours. In these cases the bacilli which have been ingested are not destroyed, and retain their normal appearance and staining reactions throughout. Here it is obvious that the first line of defence, the rapid initial phagocytosis, has been unsuccessful, and that the secondary reserve forces have not had time to come into action. It is specially pointed out by Buxton and Torrey that in these cases the invasion of the fibrin by the polynuclear leucocytes does not occur, a fact strongly confirmatory of the view that it is these cells that give rise to the opsonin or complement, the deficiency in which brings about the lethal issue.

Here we must conclude a short and altogether inadequate consideration of a subject of the highest importance. Much more might be written on the subject, but the very fact that the phenomena do not lend themselves to a brief discussion only shows how very imperfect is our present knowledge of the events which actually take place in the juices and tissues during the

process of recovery from an infective process. The mechanisms themselves are now fairly well known, the nature of the substances concerned therein thoroughly investigated, and their sources ascertained with some degree of probability; but when we come to apply our knowledge to the actual events of the living body, our difficulties begin in earnest. It is in this field of research that future advances are most to be expected.

In conclusion, let us emphasize the enormous importance which we have been led to attach to the leucocytes in the struggle against the infective diseases. In all probability the substance we call alexin, thermolabile opsonin, or complement, and which, in one or other of its actions, constitutes the first line of defence, is formed by the polynuclear leucocytes. In itself its action is but slight, and it requires to be supplemented either by the leucocyte itself, in case the reaction is mainly phagocytic, or by the action of amboceptor, which we believe to be derived from the masses of leucocytes known as lymphoid tissue. Again, the bacterium may give off toxin, especially if it has escaped being taken up by the leucocyte, and in this case these cells exhibit another phase of their protean activities, for that they can actually absorb toxin in solution appears quite certain. If this action fails the last resource is the neutralization of the toxin by antitoxin; and although this cannot be regarded as definitely proved, there is at least some reason to believe that this may be formed in the lymphoid tissues. Lastly, certain facts would seem to show that it is quite likely that even the compound of toxin and antitoxin is by no means inert unless it has been taken up by the leucocytes, and that the action of the latter substance is simply to prepare the former so that these cells may attack it. In every phase of the struggle against the bacteria the leucocyte appears, on certain or presumptive evidence, as the particular cell to which the defence of the body is entrusted.

## CHAPTER XIV

### PRACTICAL APPLICATIONS

#### Staphylococcic Infections.

COMMON as is disease due to the staphylococcus (boils, carbuncles, pustular acne, osteomyelitis, etc.), our knowledge of the inner mechanism of the pathogenicity of this organism is still somewhat scanty. The only toxins definitely known to exist are a staphylo-lysin and a leucocidin. The former is produced in three or four days in faintly acid broth, and reaches its maximum in about fourteen days. It is destroyed by a temperature of  $56^{\circ}$  C., and in other respects resembles the true toxins. Many animals, and notably the horse, contain a natural antistaphylolysin, which neutralizes the action of this hæmolysin, and is apparently a true antibody. It is present in human serum, and may perhaps account for the fact that anæmia is not a striking feature of staphylococcic diseases. Animals from which it is absent, such as the rabbit or goat, can be made to produce it by immunization with solutions of the lysin. Leucocidin is produced under the same conditions as the hæmolysin, and the two are usually produced side by side; but they are distinct substances, the former being the more easily destroyed by heat. Its action is not entirely specific: it kills the leucocytes and dissolves them, but has also some action on the ganglionic and other cells. As in the case of the hæmolysin, a natural antibody exists in the serum of man, the horse, etc., and can be prepared from other animals.

These substances, especially perhaps the latter, may play some rôle in the production of disease, in that they may inhibit phagocytosis by a poisonous action on the leucocytes. But there is little doubt that there is some other toxic body, probably an endotoxin, since young cultures killed by heat, as used for vaccines, are decidedly toxic, causing local irritation, and, if used in large doses, a rise of temperature. These vaccines contain no hæmolysin or leucocidin.

*Immunity.*—The resistance against staphylococci appears to be almost entirely dependent on phagocytic action. Ordinary methods fail to demonstrate any bactericidal action, either in normal or immune serum. Andrewes and Gordon, however, by the use of special methods, have succeeded in showing that such an action, though very slight, occurs even in the serum of normal animals, and to a greater extent, though still but slight, when the animal has been immunized. Perhaps the antibodies to the soluble toxins mentioned above may have some protective action.

The staphylococcus is very susceptible to phagocytic action, and there is a general correlation between the opsonic index and the stage of evolution of the lesion, as shown in Fig. 64. The opsonic index is greatly raised in the serum of immunized animals, and it would appear that injections of dead staphylococci have the power of increasing both the thermolabile and thermostable opsonin. The duration of the immunity is not definitely known, but is certainly short.

*Diagnosis.*—This is made entirely by the demonstration of the infective organism—an easy task. In a few cases the opsonic index may afford some help, being usually low during the acute stage.

Agglutinating sera can be prepared, but their action is not powerful, and this reaction is useless in diagnosis.

*Treatment.*—Antistaphylococcic sera have been prepared, but the results of its use are not encouraging, and the only valid method is the use of vaccines. These should be prepared either from virulent cultures derived from severe boils or carbuncles or from the lesion it is desired to treat. In practice it is a good plan to commence with a stock vaccine prepared from a virulent culture, and to prepare an autochthonous vaccine for use if the first does not succeed. In general opsonic control is not necessary, and the doses may vary between 100 and 1,000 millions, repeated every ten or fourteen days.

### Streptococcic Infections.

The toxin of the *Streptococcus pyogenes* is still not definitely known. We have already referred to the hæmolysin which it produces. There is some reason for believing that this substance has some clinical action in acute infections, and that it is produced to a greater extent by virulent than by non-virulent cultures,

Apart from this, the existence of a soluble toxin is doubtful. Filtered broth cultures of the organism are poisonous, but most observers have been able to produce immunity therewith, and the toxic substances are probably merely simple metabolic products. Parascandalo, it is true, claimed to have been more successful, but his results have not been corroborated, and there is some reason to believe that the filtrate which he used was not free from bacteria, living or dead, and that his animals were really vaccinated with a vaccine. The toxin is probably an endotoxin, though of this but little is known. The bodies of the bacteria are highly irritating, and very small doses of vaccines have to be given at the commencement of the treatment.

*Diagnosis.*—This is made by the demonstration of the micro-organism in all cases. Agglutinating sera may be prepared, but the appearance of the property in the serum in human disease is not sufficiently marked or constant to be of value.

*Immunity.*—Human blood appears to contain an antilysin which neutralizes the action of streptocolysin, and it is quite possible that this substance may play some part in the defence of the body against infections. There is also a little evidence for the formation of a true antitoxin against the actual and efficient toxin of the streptococcus, whatever this may be. This is the fact that in some cases, though unfortunately not in all, the injection of anti-streptococcic serum is followed by a marked immediate benefit in cases of septicæmia, etc. The temperature may fall, the delirium and headache pass off, and the pulse improve in a striking manner within an hour or two of the injection. Now Wright has pointed out that but little is known of the mode of action of the serum, and that it may contain a toxic ingredient and really be a vaccine in disguise. If this is the case we should not expect it to produce good effects so early as actually happens in favourable cases, in which its action resembles that of diphtheria antitoxin, and certainly suggests that some poison is neutralized. Probably, therefore, acquired immunity to streptococci is partly antitoxic or anti-endotoxic.

Bacterial immunity is probably mixed, partly bactericidal or bacteriolytic and partly phagocytic. The serum of normal animals has usually some bactericidal action, and this can be raised by immunization to a high figure; further, the serum of an immunized animal is a powerful agent in conferring passive immunity, a property not belonging (apparently) to sera of high



opsonic value. But probably phagocytosis is of the main value in the struggle against the streptococcus in the body. The organisms are very readily taken up by the leucocytes in presence of serum, and are frequently seen inside the pus cells in suppurative diseases. In general the opsonic index may be taken as a fair index of the degree of immunity, since it is usually low during the course of the infection and rises as cure is brought about. This has been already shown in the case of erysipelas: the index returns to normal in one to three days after the rise, indicating that the immunity in this case is but transient (see Fig. 63). This is of interest in view of the frequency with which this disease is recurrent. I have seen a case in which attacks occurred every three weeks for a year. But the solid immunity conferred by vaccination and the use of massive cultures is more lasting, due probably to the formation of immune bodies and thermostable opsonins.

Local immunity probably also plays a part of great importance in streptococcic infections, as shown by the fact that the erysipelatous lesion usually spreads at one margin and heals at another simultaneously. Yet even here the spread may be due to failure of access of the blood to the bacteria. This is suggested by the frequent success of the local use of iodine and other rubefacients in arresting its spread. A similar effect may sometimes be seen in chronic cases by the use of citrates or one or other of the means suggested by Wright for lowering the coagulability of the blood. A case in which the disease had been present for five weeks recovered in two days when treated in this way.

*Treatment.*—Protective treatment is not employed. It is, however, interesting to note a fact pointed out by Sir James Paget many years ago. Pathologists are as a rule more or less immune to septic infections, and these comparatively rarely occur in those who are in the daily habit of performing post-mortem examinations. The attack which proved so nearly fatal to him occurred after a long absence from the post-mortem room. Probably most pathologists are vaccinated against the disease.

The curative treatment (apart, of course, from that of the local lesion, if any, and the use of general toxic and sustaining measures) resolves itself into the question whether a serum or vaccine should be used. This question cannot be definitely settled at the present time, and the recommendations which are

given here may require great alteration in the future. In several and rapidly progressing septic cases, such as those due to post-mortem wounds, etc., the use of serum is indicated, and offers the best chance of success. In view of the very great benefit which is frequently derived from this measure, it appears highly improper to wait until a vaccine is prepared. This should be taken in hand at once, so that a homologous vaccine may be ready if subsequent events suggest its use, but in the meantime serum should be employed. The use of minute doses of a stock vaccine (5 to 10 millions per dose) may be considered, and there appears to be no reason why they should not be given along with the serum. If practicable, opsonic estimations should be taken, but the streptococcus is not as a rule a very satisfactory organism to work with. In the absence of such observations, doses of the size mentioned above may be given every three or four days.

In chronic septicæmia or ulcerative endocarditis of streptococcic origin the use of vaccines offers more prospect of success, though even here cures have been brought about by means of the serum, which should be used whilst cultures are being taken from the blood and a vaccine prepared. At present it seems desirable to use careful opsonic control in cases of this class. An excellent illustrative case treated by Douglas should be referred to for details. Here the doses were 5 to 12 millions, and the injections were given each time the index fell. The case was certainly one of septicæmia, but the evidence for the existence of ulcerative endocarditis is not conclusive.

In chronic local inflammatory disease of streptococcic origin vaccine treatment is probably the best, but here ordinary methods (especially Bier's) may be of more advantage. In the absence of opsonic control, the doses may begin at 10 millions and rise to 100 millions, and be given once a week.

Erysipelas does not usually require specific treatment, and in severe cases serum often answers well. The use of vaccines has not been tried on a sufficiently large scale to allow us to judge of its value. A case under my care of recurrent erysipelas, in which attacks had occurred about every three weeks with some degree of regularity for a year, was apparently cured by a few doses of vaccine, commencing at 25 millions and rising to 50 millions, and administered once every ten days. In some chronic cases of erysipelas it is probable that there is a considerable amount of hæmic immunity, but the protective substances are unable to

reach the bacteria. In these the use of citrates or citric acid (as recommended by Wright) is occasionally of great value.

The mode of action of antistreptococcic serum is still quite uncertain, and its use is purely empirical. Probably in most cases it acts as a bacteriolytic substance. Wright has suggested that it may contain free toxin, and so act as a vaccine; but the fact that it will passively immunize animals treated with it is adverse to this supposition. It is prepared by gradually immunizing horses and other animals until they can withstand large doses of the living organisms. The differences between the various brands mainly arise from the nature of the culture used for the purpose. The earlier sera to be prepared were procured by the injection of a single strain of streptococcus isolated from a case of erysipelas, puerperal fever, etc. It was found, however, that this serum was not successful in every case, and attempts were made to improve it. Starting from the supposition that the reason for the non-success was the fact that the streptococcus used was not exactly the same variety as that causing the infection in the patient, *polyvalent* sera were prepared by treating horses with cultures from many various sources, and it is sera of this nature that are now in general use. Another explanation was that the cultures used were not of sufficient virulence, and as antibodies to an attenuated organism may not act against the same culture when the virulence is exalted, strains of great potency were obtained by means of passage through rabbits. Yet a third method has been adopted, based on the fact that an organism which is highly virulent for one animal may be harmless to another species. Here passage is avoided, and the cultures used in the immunization of the horse are taken direct from human disease, and are used soon after isolation—*i.e.*, before virulence has been lost by prolonged cultivation *in vitro*. There is no very clear evidence that any special advantage attaches to any of the sera thus prepared; in any given case it is a matter of chance which sample will prove successful. The criterion as to the suitability of a given sample of serum is purely an empirical one. The patient's pulse, temperature, nervous condition, etc., should be watched with the closest attention, and any definite improvement occurring within the first twenty-four (and usually within the first four) hours of the first doses should be an indication to continue with the use of the same brand of serum. In some cases each injection causes a slight but definite rise of

temperature, and the symptoms undergo a brief exacerbation, followed by an improvement in the general condition. The meaning of this phenomenon is somewhat doubtful, Wright holding that the rise is due to the presence of toxin in the serum. It seems, however, more probable that it is due to a solution of some of the streptococci and consequent liberation of endotoxin. It is not a contra-indication to the use of the serum, though it may suggest care in its use. If no obvious result follows the use of the serum, the brand should be changed, another specimen being administered forthwith.

Antistreptococcic serum is, as a rule, not standardized, and the initial dose should not be less than 10 c.c., whilst 20 to 25 c.c. is not too much. Subsequently 10 c.c. may be given each day as long as it appears to be of benefit. In a generalized infection it seems reasonable to give the injections intravenously, but there is no proof that the method is of more value than the ordinary process of injecting it into the cellular tissue of the flank.

Good results have been obtained in severe cases of scarlet fever by the use of serum obtained by the use of streptococci obtained from scarlatinal throats (*S. conglomeratus*). It appears tolerably clear in this disease that a homologous serum is of advantage, the results being better than those got by the use of ordinary anti-streptococcic serum.

### **Pneumococcic Infections.**

The marked remote toxic symptoms frequently met with in pneumonia would suggest that the pneumococcus forms an exotoxin, and there is a certain amount of experimental support for this supposition. The Klemperers made use of filtrates in their experiments in the production of an antitoxic serum, and found them fairly toxic, and possessed of undoubted immunizing powers; and similar results have been obtained by Washbourn, Isaëff, and others. But the potency of these filtrates is slight compared with that of a true exotoxin, and the results obtained are quite consistent with the supposition that they are due to slight autolysis and liberation of an endotoxin. The fact that lesions occur remote from the lung cannot be taken to suggest that an exotoxin is produced, since we know that in pneumonia considerable numbers of cocci make their way into, and are destroyed in, the blood-stream. Vaccines of the dead cocci are but slightly toxic.

According to Casagrandi, non-pathogenic varieties of the pneumococcus form a hæmolyisin, whilst virulent ones do not. The same observer states that certain cultures produce a leucocidin.

The nature of the *immunity* to the pneumococcus, and in especial the causation of the crisis (when present), has been much discussed, and now seems to be fairly well elucidated. It is doubtful whether any bactericidal substances are formed in man after an attack of pneumonia; they may be produced in rabbits as a result of artificial immunization, but are not invariably present, and it is useless to attempt to explain the characteristic crisis by their agency. Anti-endotoxin may perhaps be formed artificially, but its presence in human disease is very doubtful. It is, however, now tolerably clear that recovery in pneumococcic infections is brought about mainly by the action of opsonins, and more especially thermolabile opsonins. This was well shown by MacDonald, whose investigations have been referred to already. The opsonic index is low during the course of the attack, and rises suddenly to normal at or near the crisis. Eyre distinguishes three types of opsonic index in different forms of pneumococcic infections. The first is the pneumonic type, his results precisely corroborating MacDonald; the second is the form in which recovery takes place by lysis, in which the rise is gradual; and the third, in which the disease progresses to a fatal termination, is characterized by a steady fall in the index. Eyre holds that the resistance of the individual may be measured by his opsonic response, as represented by these three curves.

The opsonin present in natural disease is, as mentioned above, mostly thermostable; but in the immunized sera of hypervaccinated animals thermostable opsonin, the bacteriotropin of Neufeld and Rimpau, is present. In all probability it is to these substances that antipneumococcic serum owes its effects. These are not great—according to Eyre, 1 c.c. of the most powerful serum yet obtained will only protect against some 300 lethal doses—as we should expect to be the case if the immunity conveyed were due to a rise in the opsonic value of the blood.

Agglutinins are usually found in cases of pneumonia, but they are not present in large amount (the serum rarely clumps at a greater dilution than 1 : 60), though a much more powerful action may be obtained by artificial immune serum. The reaction is of little value in diagnosis, since it may be absent even in pneumonia,

and is often absent in the more localized infections. The disease is recognized by the demonstration of the diplococcus.

In acute pneumococcic infections (pneumonia, septicæmia, peritonitis, etc.) attended with severe constitutional disturbance the use of serum may be tried. Several have been prepared, the methods used differing somewhat in the different cases; but, with the exception of that prepared by the Klemperers, the animals used are immunized by the use of dead, and subsequently of living, cultures. The best known are Pane's and Römer's. The latter is markedly polyvalent, an important point, since various strains of pneumococci probably differ largely *inter se*. This was very well shown by Washbourn and Eyre, who found Pane's serum protective against four cultures from different sources, but powerless against a fifth.

The results of the use of antipneumococcic serum have not been such as to lead to its general use, although several observers have reported very good effects. According to Tauber, the temperature falls to normal after one to three injections of 20 c.c., and there is marked mental and general improvement. Stress is laid on this by other physicians, and it may perhaps be due to the cutting off of the supply of endotoxins brought about by the ingestion by the leucocytes of the pneumococci after opsonization by the added serum.

The results are sufficiently encouraging to lead us to hope that the serum may become of more value in the future, possibly as a result of the discovery of a method by which the dosage and spacing of the injections and the time for bleeding may be more scientifically determined.

In general, the use of serum for localized lesions does not appear to be of much value; but great benefit has been claimed to follow the use of Römer's serum in *ulcus serpens* of the cornea, used either alone or, as Axenfeld recommends, in conjunction with a vaccine. The serum is to be dropped into the conjunctiva or injected beneath it.

Vaccines have also been used in pneumonia and in pneumococcal septicæmia with good results. Thus Eyre has reported a case of the latter disease in which there were metastases (purulent) in the subcutaneous tissues, the hip, the gluteal muscle, etc., and which recovered after five injections. In another case Eyre notes, what is of great importance, that no benefit whatever was derived from a stock vaccine, and it was only when

the patient's own culture was employed that improvement began. It is in general advisable to treat the case with a stock vaccine, and to commence to prepare a special one if this should fail. In prolonged cases it is sometimes of benefit to prepare a fresh vaccine after a time, in order to counteract any possible change of type of the organism in the body.

As a rule, the first dose should not exceed 50 millions for an adult and 10 millions for an infant, and, if the disease is febrile and the symptoms acute, may be decidedly less. Subsequent doses may be larger, but as a rule it is not necessary to exceed 200 or 250 millions. Probably the opsonic control is of more value here than in most diseases, but in the absence of this doses may be given every week or ten days. The opsonic control, it may be noted, is of more value in acute cases than in chronic ones, since in the latter the index may be persistently normal or high, and yet the disease yields readily to treatment. Thus a case of pneumococcic empyema of the frontal sinus of four years' duration, which had been twice submitted to operation, had an index well above normal, and was completely and permanently cured by four injections of a homologous vaccine at intervals of a fortnight. As a general rule, for the treatment of a small lesion in an adult, such as an unhealed sinus from an empyema, the injections may be 25, 50, 100, and 200 millions, with an interval of a week between the first two and ten days between the remainder, of course subject to any indications which may be derived from clinical observation. It need scarcely be pointed out that but little benefit can be expected in cases in which there is a mechanical obstacle to the escape of pus or the closure of an abscess. A case of very chronic pulmonary abscess, due to pneumococci, in which the X rays showed a considerable amount of thickening, derived no benefit from a long and careful course of vaccine treatment, with and without opsonic control.

Vaccine treatment has been used in acute pneumonia (according to Allen, routine injections of 25 millions may be given), and is of especial value in unresolved consolidation, which often clears up after its use in a most satisfactory manner, the moist sounds clearing up in a very short time, and the general condition improving rapidly.

Space forbids mention of all the conditions in which the pneumococcus, perhaps the most protean of all bacteria in its pathogenic effects, has been combated by vaccine-therapy.

Special mention should, however, be made of such serious conditions as *ulcus serpens* and other diseases of the eye, in which good results have been obtained by Allen and others. In general, all localized pneumococcic diseases should be subjected to vaccine treatment, if not easily amenable to surgical operation.

### Gonococcic Infections.

Here again we have to deal with an organism which exerts its pathogenic effects mainly or entirely by means of an endotoxin. The lower animals are entirely refractory to infection with living cultures, whereas the dead bodies of the cocci produce local inflammatory changes (peritonitis, etc., according to the region into which the inoculation is made), or death if the dose is sufficiently large. The toxicity, however, is but slight, and that of the filtrate from the cultures is extremely small. Several observers claim to have produced a soluble exotoxin, but there is considerable doubt as to the interpretation of their results, and in all probability the substances present in these fluids are simply traces of endotoxin, or free receptors let loose by the autolysis of a few of the cocci. Analogy with other diseases would rather suggest the absence of a powerful exotoxin. In the great majority of cases the disease is a purely local one, and the symptoms of a general intoxication very slight. The endotoxin is said to be very stable, resisting the temperature of boiling water for some hours.

The hæmic indications of immunity are but slight. Bacteriolysis has not been demonstrated,<sup>1</sup> but there is some evidence to think that immunization may be due—at least, in some cases—to a rise in the opsonic index, and since some part of the newly-formed opsonin is, or may be, thermostable, it is possible that some small amount of immune body may be produced. According to Allen, the opsonic index in acute gonorrhœal infections is somewhat below normal—0·6 or 0·7; and when spontaneous cure takes place it rises gradually, going as high as 1·6. If, however, it remains subnormal, the case passes on into one of intractable gleet. But in acute gonorrhœal conjunctivitis in adults the index may be as high as 2·5, showing, as has been already seen in other diseases, that a high index is no proof of immunity. Agglutinins also occur in the serum of immunized animals (Torrey treated a rabbit the

<sup>1</sup> Torrey has recently shown that an antigonococcic serum which he has prepared possesses powerful bactericidal properties.



serum of which clumped at a dilution of 1 : 700,000), but this does not occur in man, and the reaction is useless in diagnosis. An interesting point brought out by Torrey's studies is that gonococcic cultures exhibit well-marked differences *inter se*. The serum of the rabbit alluded to above, which clumped its homologous culture at 1 : 700,000, clumped another culture only when diluted fifty times or less. This is of importance in connection with the preparations of vaccines and sera. Torrey also showed that antigonococcic serum contained a bacterio-precipitin.

Clinical facts would suggest that the *immunity* to the gonococcus is of very peculiar nature. Thus, as Ricketts well points out, recovery is not due to a loss of virulence of the cocci, for they remain potent to produce infection during all stages of the disease. Nor is it due to the production of acquired local immunity, unless, indeed, this is of such a nature that it can be very easily broken down; for the patient can be reinfected immediately after an attack, or whilst the disease is in a chronic stage in some part of the urethra or its diverticula. It is conceivable that the gonococcus is very easily modified by passage through other human beings, and so altered that it is able to reinfect a person who has just recovered from an attack caused by it in its unaltered form; but this would hardly explain the recrudescence of an apparently cured discharge after excessive indulgence in alcohol. Even the relationship between phagocytosis and recovery is not easy to make out. Unlike other organisms, the gonococci do not appear to undergo the usual morphological changes indicative of intracellular digestion (loss of staining power and of sharp outline) usually seen in bacteria after phagocytosis; nor do the leucocytes which have taken them up show degenerative changes (Ricketts). And the fact that the gonococci are practically all intracellular from a very early period in the disease—indeed, whilst it is in active progress—would seem to indicate that it is a most inefficient protective mechanism. Further, there is no obvious difference in the phagocytosis according to the height of the opsonic index, which would lead us to believe that, even when the index is low, there is sufficient opsonin to enable all the available cocci to be taken up. As far as it goes, the evidence leads us to think that the process of cure is due to some local change—possibly to some exhaustion of a necessary nutrient material—and not to a general hæmic reaction.

Natural local immunity is very marked in the case of this

organism. A spread of the disease beyond the mucous membrane of the urethra is extremely rare. Less rare, though still uncommon, is extension to the bladder. Hæmic infections are also rare, but do occur; in them, as in other general infections, it is difficult to see how the cocci can run the gauntlet of the leucocytes.

*Diagnosis.*—In most cases, of course, this is made by the demonstration of the specific coccus—usually an easy task. If no material is forthcoming (as in gonorrhœal arthritis, etc.), two methods are available—the opsonic index and the absorption of complement. According to Allen, the normal index ranges between 0·8 and 1·2, and in chronic infections it may be low (down to 0·2) or high (up to 2 or more). Further, a dose of 75 millions of dead cocci causes but little disturbance in a non-gonorrhœal patient, but a marked rise if the patient is infected. The method of absorption of complement has been employed with marked success by Meakins, and appears to be of great value. The difficulty of the technique, however, renders it much less easily available than the opsonic method.

*Treatment.*—The use of serum need hardly be discussed. Some cases, it is true, have apparently been benefited, but it is always possible that the serum may have contained specific toxic bodies which acted as a vaccine.

The vaccine treatment, on the other hand, is of the greatest possible value. The dose varies between 5 and 500 millions. In general, large amounts are not well tolerated, especially early in the treatment. The doses may, of course, be regulated by the opsonic index, but this is probably not necessary. In acute cases two or three doses of 50 millions may be given; but it is in the chronic infections, especially, perhaps, in gonorrhœal arthritis, that the method is of especial value. Here the dose may begin with 50 millions, rising to 500 millions, or even twice this amount, and the intervals may be seven to ten days. If no benefit is obtained a further course of treatment under opsonic control may be administered. The results are usually beneficial in the extreme.

Gonorrhœal conjunctivitis is to be treated on the same lines as acute arthritis, but here, of course, ordinary local antiseptic treatment is all-important. Gonorrhœal iritis is treated like gonorrhœal arthritis, and the results are usually excellent.

### Meningococcic Infections.

Very little is known concerning the toxin of the meningococcus. Cultures are of very feeble toxicity for animals, and large doses of the vaccine are usually (though not invariably) well tolerated by human patients. It is probably an endotoxin which is only produced under certain conditions. Its effect in the human subject is mainly a local one, manifested chiefly on the tissues in or near which the cocci are localized. The disease is in most cases a local one, the organism being rarely found in the blood or organs other than the brain and cord.

The frequency with which the organism is found within the polynuclear leucocytes would lead us to believe that the organism is combated in the main by phagocytosis; and this is confirmed on the whole by the results obtained by a study of the disease by Wright's method. When opsonic determinations are made using a virulent culture, such as one derived from the patient himself, it is found as a rule to be taken up very badly in preparations made with normal serum and leucocytes, and very well when some of the patient's serum is present, so that very high indices are obtained. This is obviously because the cocci have become animalized, like the virulent pneumococci studied by Rosenow and others. They require a large dose of opsonin, such as is present in serum from a patient who is attempting to combat the disease, and not in that from a normal person. The result is that the opsonic index of these patients is often extremely high, figures of 10 or more being common, and 40 has been recorded. Houston has pointed out that old laboratory cultures which have lost their virulence are phagocytosed more easily in presence of normal serum, so that the opsonic index of meningitis patients, as determined by the use of these non-virulent cultures, is comparatively low. Houston has proposed this test for distinguishing between the "true" meningococcus and allied cocci of similar morphological characters. He determines the opsonic index of a patient suffering from true cerebro-spinal meningitis against a normal control, using both a known meningococcus culture and that under consideration. In what he terms the positive reaction there is, in the case of the normal blood, very little phagocytosis, and no agglutination of the cocci which are not ingested, whilst with the blood from the cerebro-spinal case there is much phagocytosis and marked clumping of the free cocci. It would seem,

however, that the test is simply one of the virulence of the culture, rather than that of its specific nature. As regards agglutination, Houston and Rankin point out that this property also is lost on prolonged cultivation on artificial media; and as regards the behaviour of the coccus in opsonic estimations, exactly the same phenomena are seen when cultures isolated from what are apparently ordinary cases of basic meningitis are tested against the blood of the patient and a normal control. It seems more reasonable to suppose that the coccus from the basic meningitis cases are either of lower virulence, or that their virulence has developed along different lines from a common non-virulent stock, rather than a different species.

The opsonin present in the serum in meningitis is to a large extent thermolabile. Some thermostable opsonin is present, the amount being roughly proportionate to the height of the index.

Agglutination is somewhat difficult of study in the case of this organism, owing to the variations presented by different cultures in this respect and the difficulty in procuring a homogeneous emulsion. Tested by ordinary methods, the blood of meningitis cases does not clump in high dilutions: according to Davis, 1 : 50 is about the average. Kutscher states that the phenomenon is much more marked at 55° C., and that a culture which was not agglutinated at all by a specific serum at 37° C. was clumped in twenty-four hours at 55° C. in dilutions of 1 : 500, or even 1 : 1,000. If this is correct it may prove important in the clinical diagnosis of the disease, which at present is based mainly on the characters of the cerebro-spinal fluid, and especially on the presence of the coccus. The cytological and chemical examination of the fluid affords definite evidence of the presence of a meningitis, but the cocci are not always discoverable, especially in cases of internal meningitis, or those in which the foramina at the base of the brain are closed. The opsonic index may be of great value, especially if a virulent culture is at hand. According to Houston and Rankin, the positive "reaction" described above is not usually present before the fifth or sixth day in the epidemic form.

The serum of immunized animals contains a substance which gives the phenomenon of fixation of complement when combined with meningococci, and this fact is used by Kolle in the standardization of his therapeutic serum; but whether this is a bacteriolysin or bactericidal substance is not definitely known. According to Davis, normal blood-serum is bactericidal to menin-

gococci, and this power is increased in meningitis. He states, however, that the opsonic power of the blood was not altered during the disease.

The treatment differs in the acute and chronic stages. In the very acute cases the only hope appears to be in the use of a serum, coupled of course with the more ordinary remedial measures, such as repeated lumbar puncture. This probably acts by removing the inert cerebro-spinal fluid (which is almost deficient both in opsonin and in complement), and causing the exudation of fluid containing a larger amount of protective substances. In more chronic cases, and especially in posterior basic meningitis, the use of vaccines, either alone or in conjunction with serum, is more promising.

Several sera have been prepared—Kolle and Wassermann's, Jochmann's, Ruppel's, Flexner's, and Burroughs and Wellcome's—and very diverse reports have been published concerning their value. Small differences exist in the methods of preparation, but in all cases large doses of organisms, dead or living, are injected, either subcutaneously or into the veins. The potency of the serum is tested either by agglutination or by the absorption of complement. According to Houston and Rankin, some of the therapeutic sera in common use have very slight opsonic power and are devoid of agglutinating properties. It seems quite clear that the serum is useless if given hypodermically, and the observers who have obtained beneficial results (Flexner, Levy, and others) have injected the serum into the spinal canal after removing an equal amount of cerebro-spinal fluid by lumbar puncture. The dose is 20 to 30 c.c., repeated several times at intervals of twenty-four hours. Some patients (adults) have received as much as 340 c.c. The injections may cause vomiting and unconsciousness, but no permanent inconvenience; they are painful, and this is attributed to the use of carbolic acid as a preservative agent. The treatment must be commenced early, and in view of the results of Levy, in whose practice the deaths fell from 78.57 per cent. to 6.25 per cent., and Flexner, who reduced the mortality to 20 per cent., seems to be of decided value. Other observers have, however, been less successful.

The use of serum from convalescent cases of meningitis (which they find to possess marked bactericidal properties) has been suggested by MacKenzie and Martin. The blood is collected by venesection, whipped, and centrifugalized, and the serum used

for intraspinal injection. In some cases the blood used was taken from the patient himself, and they obtained encouraging results after the use of both methods. The method is perfectly rational, and we might expect on a priori grounds that fresh serum, containing its full amount of complement and thermolabile opsonin, would be of more benefit than stale immune serum. With the idea of causing an increased outflow of preventive substances from the patient's blood by producing a mild aseptic inflammatory process, Briscoe has injected dilute carbolic acid solutions and other fluids into the cerebro-spinal canal, but without success.

The treatment by vaccines has not attracted much attention, but in the subacute and chronic cases it is well worthy of a trial. I have used it in four cases, with three recoveries and one death. These figures are not sufficiently great to argue about, but what was most obvious was the very decided clinical improvement which followed almost every dose. This occurred too frequently to be mere coincidences, and as these patients were all young children the question of their being due to a mental effect need not be considered. The dose has been 250 millions, increasing to 500 millions, and 1,000 millions, and no bad effects have been noticed. They have been usually regulated by opsonic control, and the improvement in the general condition as the index rises appears to me to be more definite in this disease than in most others. It need hardly be pointed out that no form of specific treatment will cure the obliteration of the foramina in the roof of the fourth ventricle and consequent hydrocephalus, which is so frequently present in these chronic cases.

In some cases the index shows a very marked rise (to 10 or more) as a result of a single injection of a homologous vaccine, whilst in others the reaction is much less, and the level does not rise much above unity. It is probable that these two types of reaction correspond to infections with the cerebro-spinal and basic meningitis types of organism, but the cases show no obvious clinical difference.

### **Malta Fever.**

The toxin of Malta fever appears to be an endotoxin. Killed cultures are decidedly toxic for animals, and their prophylactic use in moderate doses has been followed in some cases by the development of chronic aseptic abscesses (Eyre, Bousfield). The type of immunity is not known; no bactericidal substance is

known to be developed in the blood, and Eyre's attempts to produce a powerful curative or preventive serum were unsuccessful. There is more evidence pointing to opsonic action in the cure of the disease and the subsequent immunity: the index early in the disease is as a rule low, and it rises during the progress of the case. In some points the immunity reactions of the disease resemble tubercle (slow development of the immunity, frequency of relapses, absence of known bacteriolytic properties in the serum, local toxicity of the cultures), but there is this marked difference, that Malta fever is essentially a septicæmia, the cocci being present in the blood in small or large numbers in practically all cases.

The agglutinative reaction, first studied by Wright and Smith is of enormous importance in the diagnosis of the disease, and it was Zammit's discovery of clumping powers in the blood of the goat that led to the discovery of the fact that the milk of these animals was frequently infective even when the animal had no signs of disease. Remedial measures based on this phenomenon have been of enormous advantage in checking the disease.

Various criteria are adopted in the application of the reaction to diagnosis of disease in man. Critien uses a dilution of 1 : 10, and a time-limit of half an hour, and finds results thus obtained as conclusive as if higher dilutions were used. Bassett-Smith recommends 1 : 30, with a time-limit of four hours, or twenty-four if the macroscopic method be used. Eyre, however, points out that the blood not infrequently clumps in a high dilution, but not when more concentrated—*e.g.*, giving no reaction between 1 : 10 and 1 : 100, but clumping strongly at 1 : 200. It appears, therefore, that a series of dilutions should be made if negative results are obtained with the test adopted as a standard. Agglutination may be manifested in very high dilutions, even as high as 1 : 500,000. It usually appears about the fifth day. The only other certain method of diagnosis is the isolation of the organism from the blood, usually a fairly easy matter.

The treatment of the disease by means of vaccines has been studied by Reid, and more recently by Bassett-Smith. The latter recommends a ten-day-old agar culture, emulsified in distilled water, sterilized at 60° C. for an hour, and sufficient carbolic acid added to bring the strength to 0.5 per cent. It is standardized by drying 20 c.c. (of course, before the addition of the carbolic acid), and weighing the solid residue. The results thus obtained

are used to determine the degree of dilution necessary, so that 20 c.c. of the vaccine should contain 4 milligrammes of solid substance. The doses given were between 0.25 and 0.5 c.c. They were checked by opsonic control twice weekly, and were in general given once a week or fortnight. Bassett-Smith does not approve of the method in acute cases, but his chronic ones seemed decidedly benefited.

The prophylactic use of the vaccine has not been tried on a scale sufficiently large to enable any very definite inferences to be drawn therefrom. The doses may be 200 to 400 million cocci, and in general two are given, the results being judged by their effect on the agglutination reaction. As noted above, some bad local results have been noticed, and there is in all cases a good deal of fever, malaise, and inflammation at the site of injection and corresponding lymph glands.

### Tubercle.

The actual toxin and mode of action of the tubercle bacillus remain unknown. The most interesting substance which has been derived from cultures of the organism is tuberculin, which has been discussed previously. That it is not the true toxin appears from the fact that animals immunized to it are still susceptible to the tubercle bacillus, and a serum can be prepared which appears to be an antitoxin for tuberculin, but it has no curative or preventive effects in tubercle. The fatty substances which confer on the bacillus its peculiar staining properties are not devoid of toxicity. They cause chronic inflammatory and caseous changes in the tissues, and may perhaps play a larger rôle in the evolution of the lesion than is generally thought. And it must be pointed out that tuberculosis may be an afebrile disease throughout. The temperature of phthisis is mainly due to other organisms, notably to the pyogenic organisms which form such frequent secondary contaminations of the vomicae. The chief *pure* tuberculous affection in which fever is a marked and constant symptom is tubercle of the meninges, and here the local processes are in close proximity to the cerebral cortex. In general tuberculosis there is usually fever, but there is also usually a focus exposed to secondary infections, or a broncho-pneumonia in which other organisms play a part. The main toxic effect which we recognize as being due to the tubercle bacillus is exerted on the surrounding tissues, and there is no disease which is more



truly a local one ; meaning thereby that in pure tuberculosis the general symptoms indicative of a spread of the toxins into the general circulation are but slight, and the local lesion constitutes almost the whole of the disease. Note, for instance, the good general health which often occurs in patients with tuberculous glands in the neck, even if of some size. And where the general health is impaired, it will often be found to be a predisposing cause rather than a result of the tuberculous process.

*Immunity.*—These facts would tend to show, what I believe to be the case, that the immunity to tubercle is local rather than general. That general immunity exists there can be no doubt, but we must distinguish between general immunity due to a condition of the blood and that due to a resisting power inherent in all the tissues of the body. Thus to take two patients, one strong and robust and the other of enfeebled vitality : the latter may fall a ready victim to tubercle, whilst the former resists exposure to the most virulent infection : yet there may be no demonstrable difference in the serum of the two persons. There may, it is true, be a slight difference in the opsonic index, but this is not invariably the case.

The importance of local immunity in tubercle appears clearly from the fact that areas of advance and of cure may occur in the same patient, or even in different parts of the same lesion. This is often very well seen in cases of lupus, but is even more marked in sections from chronic tuberculosis—*e.g.*, of a synovial membrane, in which areas of cure (as indicated by fibrosis and organization of the giant-cell systems) and of extension (as indicated by caseation and the formation of new tubercles) may almost always be found in the same lesion. In general, tubercle tends to spontaneous cure, and when a lesion continues to spread it will often be found that some second influence (such as a secondary infection) is at work ; even in debilitated subjects there is usually an effort at spontaneous cure in some parts of the lesion.

As regards the nature of this process of cure, we can say but little. There can be but little doubt that the main curative agency is phagocytosis, but there is no reason to think that this is dependent on the same mechanism of opsonic action that we reproduce so readily *in vitro*. Polynuclear leucocytes are conspicuous by their absence from tubercles, and the only cells in which tubercle bacilli have actually been demonstrated in the

living body are the giant and endothelial cells. In these, as Metchnikoff pointed out many years ago, tubercle bacilli can be seen in all stages of degeneration, from bacilli possessed of normal staining properties to mere "ghosts." Either the endothelial and giant cells actually engulf the bacilli, or, what is perhaps more likely, they grow round them, being stimulated to growth and proliferation by the action of the same toxin which, when more concentrated, leads to caseation and death. Whether any opsonin is necessary for this process to occur, or whether this opsonin is produced locally or makes its way from the blood, we do not know. What we do know, however, is that a high opsonic index does not *necessarily* imply that the local lesions are undergoing cure; chronic tubercle, and especially lupus of some standing, is often associated with a very high index. But Bulloch found that the cases of lupus which did well on X-ray treatment (which, amongst other effects, causes a permeation of the lesions with plasma) were those that had a high index, and that in the cases in which it was low originally better results could be obtained if it were raised by means of tuberculin injections. It is, therefore, not improbable that opsonin may actually soak through the lymphoid zone and sensitize the bacilli, thus aiding phagocytosis by the giant cells. But this is by no means certain. The results of an injection of tuberculin are probably very complex, and it is at least possible that the curative effect is mainly or entirely due to the production of a local reaction in the neighbourhood of the lesion, as a result of which it is flushed with blood, and perhaps simply increased as regards nutrition. Very small doses of tuberculin are sufficient to cause a slight but definite reaction. This may be quite inappreciable in most regions, but perfectly obvious in the case of tubercles of the iris, which may be seen to become surrounded by a hyperæmic zone after injections of  $\frac{1}{1000}$  milligramme of new tuberculin, or even less. Reactions of this nature are, of course, entirely harmless, and are unaccompanied by the slightest rise of temperature, if any.

The only rôle which we can assign with any degree of probability to the polynuclear leucocyte in the struggle against tubercle has regard to its action on bacilli which have made their way into the blood. Here the conditions are much more like those which occur in our opsonin experiments, and it is quite likely that opsonization and phagocytosis occur. Even this is not certain, however, for we do not yet know definitely whether opsonin exists

in the circulating blood-plasma, and that the mechanism is of comparatively little value appears from the fact that rupture of a caseous gland into a vein is so often (as far as we know, always) followed by general tuberculosis. It is possible that living and virulent bacilli may be taken up by the polynuclear leucocytes, carried to distant organs, such as the lymph glands or spleen, and there continue to grow, producing anatomical tubercles.

As regards the nature of tuberculo-opsonin, the normal opsonin is completely thermolabile, and the rise in the index due to injections or the natural disease is mainly of that nature also. Occasionally the presence of thermostable opsonin is demonstrable, but it never becomes abundant.

We know but little concerning any other antibody or defensive mechanism. No bacteriolytic or bactericidal action is demonstrable. This may possibly be due to the technical difficulties incidental to investigations of this nature on a bacillus which owes its staining properties to the presence of fats (which we cannot expect a serum to dissolve), and which grows so slowly. Wassermann and others have shown that antibodies occur in patients immunized with tuberculin, but we do not know their nature. Nothing really definite is known concerning an antitoxin. An agglutinin is often formed, but it may be absent throughout the whole course of the disease, so that we cannot regard it as of importance.

*Diagnosis.*—Where possible this should be made by the recognition of the bacillus, an achievement which modern methods have made possible in many cases in which it would formerly have been regarded as out of the question. Into this and into questions of cytology, etc., we need not enter. The main methods to be considered are—(1) the tuberculin reaction, and (2) the opsonic index.

1. *The Tuberculin Reaction.*—The old tuberculin is used, and is best bought ready prepared, as issued with the German Government stamp. It may be standardized, but this process is uncertain, animals differing markedly in susceptibility. Several methods have been introduced, but are not in general use. Behring's method is to determine the lethal dose for guinea-pigs on subcutaneous injection, whilst Von Lingelsheim injects directly into the brain, the susceptibility of which is much greater. As a rule, a normal guinea-pig will stand a dose of 1 c.c. with impunity. It should be diluted before use with sterile normal saline solution

containing 0.5 per cent. carbolic acid or lysol, and kept in sealed "ampoules," each containing 1 c.c. A convenient method to adopt is to take 99 c.c. of diluent and add the whole of a 1 c.c. bottle of tuberculin. Each cubic centimetre of this contains 10 milligrammes of fluid, or  $\frac{1}{100}$  c.c.; several ampoules are prepared, and the remainder of the fluid diluted with an equal amount of the diluent. Each cubic centimetre now contains 5 milligrammes, or  $\frac{1}{200}$  c.c., and several ampoules are filled with this dilution. Lastly, what remains, or part of it, is diluted with four times its volume of diluent, so that each cubic centimetre contains 1 milligramme, or  $\frac{1}{1000}$  c.c., of tuberculin. It will be noted that the fraction of a milligramme refers to the tuberculin as sold, and not to any active constituent it is supposed to contain. It is recommended to err on the side of caution, and to commence with  $\frac{1}{1000}$  c.c., and then to go on to  $\frac{1}{200}$  and finally  $\frac{1}{100}$ ; but with proper selection of cases it is probable that the risk with  $\frac{1}{200}$  is infinitesimal. The rise in temperature which constitutes a reaction should be at least 1° F., and is usually more. It usually commences in eight to twelve hours, rises for another two hours, remains up for a few hours longer, and then falls rapidly. There is often a considerable amount of general malaise.

It should be used only in cases in which the diagnosis cannot be made by other methods with ease and certainty, and in which it is of great importance that it should be made definitely and rapidly. Thus, a patient living under favourable conditions who developed ambiguous indications of phthisis might fairly be watched for a time, his weight recorded, an attempt made to obtain sputum, etc. But the same signs occurring in a person who was about to get married, or a medical man thinking of taking a resident appointment in a hospital, would be a strong indication for the diagnostic use of tuberculin. Perhaps its main value is that it enables a negative diagnosis to be made with some degree of confidence, and is the only agent which will do so. No reaction after two injections of  $\frac{1}{100}$  c.c. may be taken as definite proof that the disease is absent.

It should *not* be used (1) when the temperature is irregular—obviously, since the rise might not be due to the tuberculin—and (2) when there are secondary infections.

Some cases of syphilis, leprosy, and actinomycosis are said to react, but this is unusual, and the concomitant presence of tubercle has not always been ruled out.

In *cattle* the doses are larger. The tuberculin is usually diluted

ten times, and the dose varies from 4 c.c. for a large bull to 1 c.c. for a calf under one year. The temperature is taken for a day or two before the injection, and at the ninth, twelfth, fifteenth, and eighteenth hours afterwards. The rise is gradual, reaching its maximum in twelve to fifteen hours, and then falling gradually to normal. According to Nocard, anything over  $2.5^{\circ}$  F. is diagnostic, from  $1.4^{\circ}$  to  $2.5^{\circ}$  F. suspicious, and under  $1.4^{\circ}$  F. unimportant. The temperature of the animal should not exceed  $103^{\circ}$  F. when the injection is made.

*Von Pirquet's reaction* or the *cutireaction*: This was introduced as a method of avoiding the dangers supposed to be incidental to the use of tuberculin *sub cute*. A very gentle scarification of the skin is made, just as for Jennerian vaccination, avoiding drawing blood, if possible, and the abraded surface covered with diluted tuberculin (1 part with 3 of normal saline containing 0.25 per cent. carbolic acid). A control scarification is made, preferably on the other arm, and covered with the carbolized normal saline solution. The reaction takes the form of a red papule of varying size, sometimes extending for  $\frac{1}{8}$  inch or more in all directions from the site of the original scarification. I have seen the skin so sensitive that a vivid reaction was obtained where the diluted tuberculin had accidentally run down the skin. The redness increases for a day or two, and may remain for four or five days, but in general disappears earlier. It is to be compared with the control side, which has also been treated with dilute carbolic acid, but no tuberculin. Von Pirquet now uses undiluted tuberculin, the control scarification not being treated at all. This is probably the better method, and seems devoid of danger. In Moro's method an ointment containing tuberculin is rubbed into the skin.

The process is probably quite devoid of danger. Some tuberculin is certainly absorbed, since I have seen a quite definite local reaction round a tubercle in the iris; the temperature, however, does not usually rise, but may do so; this is a sign that the scarification has been too deep. No reaction is given in advanced cases of the disease, probably because the resisting powers are too low for the production of the necessary antibodies. The same, it may be noted, is true for the ordinary tuberculin test, if it were ever justifiable to use it in such cases.

Of the value of the test there is no doubt, and a well-marked reaction is conclusive. A good deal of difficulty arises from slight and doubtful reactions, and it sometimes appears to fail in

cases definitely tuberculous. A positive reaction is here of more value than a negative one.

*Calmette's reaction* was introduced soon after Von Pirquet's, and consists in a slight conjunctivitis and oedema of the caruncle, with sero-fibrinous discharge, which occurs in tuberculous subjects after the instillation of a drop of well-diluted tuberculin into the eye. Since glycerin has an irritant action, a special preparation devoid of this substance is sold for the test. It is put up in a dry form (being precipitated by alcohol), and when dissolved in water according to the instructions gives a dilution corresponding to 1:100 or 1:200 of the original tuberculin. The strength has been gradually decreased owing to calamitous results having been obtained when the stronger dilutions were used. It should certainly never be used when the eyes are affected, and even when they are healthy I personally do not consider the risk worth running unless the diagnosis is of great importance, and all other methods have been tried and have failed.

2. *The Opsonic Index*.—Numerous observations by different observers have shown that in health the opsonic index to tubercle lies between 0·8 and 1·2, the exceptions being very rare and due perhaps to slips in the technique. As a matter of fact, these are outside figures, and in the great majority of cases the index will be found to lie between 0·95 and 1·05. If, therefore, the diagnosis lies between health and tubercle, an index below 0·8 or above 1·2 is very strong evidence in favour of the latter, becoming more convincing the remoter it is from these figures, either above or below. Indices between 0·8 and 1·2 are not of much value, yet a figure of, say, 0·85 on several occasions is very suggestive.

There is not sufficient evidence as yet to show how other diseases influence the tuberculo-opsonic index. In the majority of cases the figures lie within the normal limits, but there are occasional exceptions. In general, therefore, the results given above will apply, but the conclusions are less certain.

A rapid variation of the index, either (*a*) spontaneous, or apparently so, or (*b*) due to auto-inoculation from exercise or massage of the lesion, or (*c*) caused by an injection of a small dose of tuberculin, is much more suggestive—indeed, practically diagnostic, supposing no errors are made in the determination. And it must be emphasized that when the opsonic index is required for diagnostic purposes, the most careful technique and attention to every detail is absolutely necessary.

The *agglutination reaction* has been recommended as a means of diagnosis, but it is not always present at any stage of the disease, and there are practical difficulties in the way of its determination. It does not appear to have come into general use even in France, where much attention has been paid to it.

*Treatment.*—The essays at a serum treatment have been many, and no method has attained any appreciable degree of success. Good results have been obtained, it is true, by several, especially in the hands of their inventors, but recent work by Hort and others has shown that normal horse serum has some value as a curative agent. Maragliano's serum appears to be prepared by injecting animals with various extracts of tubercle bacilli, and is supposed to be both bactericidal and antitoxic. It is given either alone or in conjunction with a vaccine. McFarland prepared an antituberculin by immunizing donkeys for long periods with tuberculin, and found that it annulled the effects of tuberculin on tuberculous animals, but had no protective or curative powers as tested on guinea-pigs. Clinical evidence appeared to show that it was of some value.

Marmorek's toxin is quite different from any of the others, which are various extracts of tubercle bacilli, or of their soluble products. It is prepared by cultivating young bacilli (prepared in such a way that they form homogeneous emulsions) in a medium containing leucotoxic serum, prepared by injecting calves with guinea-pig leucocytes. It is supposed to be the actual toxin which is developed *in vivo*. This substance (which is of feeble toxicity) is used to immunize horses. Some good results have been obtained, but perhaps we may attribute them to minute amounts of tuberculin which may be formed in the cultures and remain unabsorbed in the blood of the horse when it is bled. The experience of most observers has not been favourable to its use.

The other sera which have been introduced do not call for further notice.

The most hopeful method of combating tubercle—other than the all-important use of fresh air, good food, and careful regimen—consists in the use of a vaccine. There are many to choose from—old tuberculin, TR, BE, bovine tuberculin, oxytuberculin, antiphthisin, etc.; but of these, Koch's preparations (old tuberculin, TR, and BE) are the only ones in general use, and appear at least as good as any.

TR (*tuberculinum residuum*) is prepared by triturating living, dry,

virulent tubercle bacilli by a mechanical process in an agate mortar. The powdered bacilli are suspended in distilled water and centrifugalized. The supernatant fluid has the properties of the old tuberculin, and is called TO (O = *ober*, = upper). The residue is dried, and again powdered, emulsified, and centrifugalized, and the bacilli are now found to have been reduced to a uniform mass. This constitutes TR, which thus corresponds somewhat to an endotoxin. It is diluted with 20 per cent. glycerin, and when diluted it is advised that no carbolic acid should be added. There has been a good deal of confusion with regard to the dosage. It arose from the fact that 1 gramme of the dried tubercle bacilli is used in the preparation of 100 c.c. of the remedy, so that each 1 c.c. of the latter contains the material derived from 10 milligrammes of bacilli, and is supposed to be equal in immunizing power thereto. But the amount of *dry residue* which it contains is only one-fifth of this amount, the fluid being standardized before issue, so that 1 c.c. contains 2 milligrammes of solid substance. The dosage adopted in this book concerns this dry residue only.

Tuberculins are also prepared from bovine tubercle (*perlsucht*) bacilli, and its use mixed with the human form (as recommended by Allen) appears rational and quite worthy of a trial. The majority of cases of human tubercle are due to bacilli of the human type, but at the worst the material derived from the bovine bacilli will be inert and do no harm.

Another substance used as a vaccine is Koch's Bazillen-emulsion, or Neutuberculin, usually known as BE. It consists of a suspension of dried and ground up bacilli in equal parts of glycerin and water. When used as a vaccine it is diluted with normal saline solution and heated to 60° C. to insure sterility. This may also be done with TR. It contains 5 milligrammes solid substance per c.c.

As regards the use of these substances: We may recognize two, or perhaps three, methods—(1) the intensive; (2) the opsonic, and (3) the uncontrolled use of small doses.

1. In the *intensive method* the idea is to bring about as much immunity as possible as quickly as possible, by the use of rapidly increasing doses, taking care always to avoid a reaction. The commencing dose is very small, about  $\frac{1}{10}$  milligramme ( $= \frac{1}{100000}$  c.c.) of old tuberculin, or  $\frac{1}{10000}$  milligramme (of solid substance) of TR. The dose is repeated two or three times a



week, and is gradually increased until 500 milligrammes ( $=\frac{1}{2}$  c.c.) of old or 2 milligrammes of TR is given. If a reaction occurs an interval of a week is given, and the treatment recommenced with a smaller dose. The dose of BE may be the same as that of TR.

There are numerous slight modifications in detail, but the foregoing outline will serve as a general description of the process as it is used in many of the Continental clinics. As a rule no attempt is made to estimate the degree of immunity, but Koch suggests the agglutination reaction for this purpose. It usually rises to 1 : 100, or thereabouts, and may go much higher.

As a result of the treatment, the patient is certainly immunized to tuberculin, since he fails to react to fairly large doses. The process is then stopped for a time and recommenced. The beneficial effects as recorded (as I have seen on a small scale) are undoubted, and the risks in careful hands and in properly selected cases appear to be slight.

2. The *opsonic method* consists in the use of such doses at such intervals as will cause the maximum increase in the opsonic index. The doses here are very much smaller than in the last method. TR is usually employed, and the amounts range between  $\frac{1}{100000}$  and  $\frac{1}{1000}$  milligramme. The idea, of course, is to avoid the possibility of a summation of negative phases, and to call forth the greatest possible formation of protective substances. It is extremely difficult to carry out, since the index alters so quickly in most cases of progressive tubercle that very frequent determinations of the index are necessary. Perhaps it is the ideal method, and all who undertake tuberculin treatment on a large scale ought to acquire some experience of it, but considerations of time will prevent its ever coming into general use as a routine treatment. In practice a sort of modified opsonic method is sometimes used. The first few doses are controlled by the index, and the optimum dose and interval determined. These are then preserved throughout the treatment, with perhaps occasional determinations of the index at times.

It must be pointed out that the theoretical necessity for the opsonic control depends on the acceptance of the fact that tuberculin exerts its beneficial influence by causing an increase in the amount of opsonins present in the blood. But this is not certain. Admitting that the immunity is due to antibodies, these may be bactericidal substances or anti-endotoxins, and we have

no reason whatever for thinking that the opsonic index affords any clue to the amounts of these substances formed. And, of course, as is held by many, if the beneficial effects are due to repeated slight local reactions, acting in a way as yet not understood, the opsonic index is of little value. It should not be forgotten that a polynuclear leucocyte will engulf an enormous number of tubercle bacilli in a short time even when the opsonic index is low, and it seems improbable that it ever falls so low (even when the negative phase is most marked) as to prevent these leucocytes from doing their work, presuming the bacilli are accessible. A high opsonic index may have some action in preventing generalization of the bacilli—*e.g.*, at a surgical operation—but even this is not certain.

It must be noted that the beneficial effects of vaccine treatment by the opsonic control are not denied, but that the opinion is expressed that the same benefits may be obtained by simpler methods.

Space forbids more than a reference to the use of auto-inoculation by graduated exercise, massage, etc. This is supposed to expel some bacilli or products thereof into the lymph spaces, etc., where they act as a vaccine. The benefits of these measures is undeniable, but it may be doubted whether it is due entirely or in part to auto-inoculation, and it would appear preferable to employ vaccines in known doses.

3. *The use of small doses without opsonic or other control.* This combines the advantages of practicability and safety, and often gives good results. How they compare with those obtained by the other methods cannot be accurately known. Probably no harm has ever resulted from  $\frac{1}{1000}$  milligramme of TR, and if this amount is given once in every ten days, or even once a week, many cases improve in a most striking manner. My experience has been mainly confined to its use in surgical cases, and though I have met with numerous disappointments (which I believe are not unknown with the most strict opsonic control), I have seen others in which rapid cure—*e.g.*, of a chronic sinus or tuberculous ulcer—has been obtained. I should not recommend it or any other method of vaccine treatment in place of surgical or sanatorium treatment, where applicable; in conjunction with other methods, it has its place.

*Preventive Treatment.*—This is at present of but little importance in human pathology. A person who is in danger of becoming

tuberculous should be removed from the infection and placed under good hygienic conditions. A few injections of TR may be given, however, and analogy with a similar procedure in cattle would lead us to believe that it may be of some value.

Attempts to immunize lower animals to tubercle have been made from a very early period; it was, indeed, the discovery of the fact that dead bacilli are absorbed with great difficulty that led Koch to devise his TR. The vaccines that have been employed are numerous—cultures of low virulence, dead cultures, avian and reptilian bacilli, etc.—but the subject entered on a new and most important phase in 1901, when von Behring published his method, which is of great theoretical interest, and from which important practical results in the stamping out of bovine tubercle (so great a pest to infants, from the prevalence of bacilli in milk) have been esteemed possible. Here the vaccine consists of living tubercle bacilli of human origin. This is of feeble virulence for cattle. It is prepared by being dried *in vacuo*, emulsified with glycerin in a mortar, and diluted with normal saline solution containing 0.15 per cent. of sodium carbonate. It is standardized in such a way that 1 c.c. = 2 milligrammes of dried bacilli. The injections are made intravenously; two are given—the first of 1 c.c., and the second, about twelve weeks later, of five times this amount.

There is no doubt that this process confers a certain amount of immunity, or rather of increased resistance. The process is harmless to calves, but sometimes kills older animals from pulmonary œdema. The immunity lasts for a year or so.

The method has been carefully examined at Melun by Rossignol and Vallée, and their results were quite favourable. The vaccinated animals were tested (along with controls) by subcutaneous injection of bovine tubercle, by injections of these bacilli into the veins, and by keeping them in a shed with animals known to be tuberculous, and in all cases a greatly increased degree of resistance was found. But Lignières pointed out a remarkable fact, that animals which have been treated in this way and which do not react to tuberculin, and are apparently normal at the autopsy, nevertheless may contain living tubercle bacilli in their lymphatic glands. This is of extreme interest in connection with the question of the latency of bacilli in the tissues.

Von Behring's latest vaccine is known as tuberculase, or tulase. It appears to be prepared by the action of chloral on tubercle

bacilli, but the exact details are not available, and the preparation is not yet obtainable. It seems not to contain living bacilli.

Attempts have been made to vaccinate against tubercle (and to treat it in human subjects) by ingestion. Some degree of success appears to have been obtained by feeding calves with living human tubercle bacilli, and some physicians have claimed good results by administering a minute dose of TR by the mouth; but in what way these methods, so uncertain as to the dose which is actually absorbed, are preferable to the use of the syringe is not very clear.

This is a very brief epitome of a field of research which would require many volumes for its adequate treatment. I have attempted to give the main essentials only.

### **Typhoid Fever.**

The pathogenic action of the typhoid bacillus appears to be due entirely to the action of an endotoxin which is set free when the bacillus undergoes solution in the body. This toxin has a local and a general action. The former is marked in the regions which harbour the bacillus, and in which it undergoes solution, either by autolysis or by the action of bacteriolysis—the Peyer's patches, abdominal lymph glands, spleen, etc. In these regions it produces hyperæmia, followed by inflammatory hyperplasia of the lymphoid and endothelial cells, which diminish the blood-supply and may lead to necrobiosis of the inflamed tissues. In the case of the Peyer's patches this commonly occurs, the lymphoid tissue being cast off as a slough, and the wound, becoming infected by intestinal organisms, may extend deep into the bowel wall and cause hæmorrhage or perforation. The process is less likely to occur in the solid organs, which are nourished by blood from all sides and in which secondary infections are less frequent. The general effects of the toxin—fever, degenerations of the kidney, liver, etc.—are not characteristic. The failure of a leucocytic reaction of the bone-marrow, and consequent leucopænia, is worthy of notice.

The fact that typhoid fever is a septicæmia and that the living organisms circulate in the blood must not be forgotten.

*Immunity.*—A short time ago typhoid fever was regarded as a disease in which the immunity was purely bacteriolytic. During an attack of the disease or the process of artificial immunization the amount of immune body (which occurs in small amounts in health) shows a steady rise, and the blood soon becomes extremely

potent in this respect. The bacteriolytic power of the blood as tested by Wright's method does not necessarily show a great increase; this is due probably to the lack of complement, and it is possible that relapses may be due to the same cause, deviation of complement occurring owing to the excess of immune body.

There is, however, no doubt that phagocytosis plays a part of the utmost importance in the natural cure of the disease, and that the opsonic content of the blood (as tested by one of the dilution methods) undergoes a steady rise during the process of immunization. We may regard the mechanism by which the infection is combated as a double one, bacteriolysis and phagocytosis being jointly concerned. That the latter is of chief importance appears probable, from the fact that typhoid bacilli set free their endotoxin when dissolved by means of bacteriolytic sera.

The antitoxin or anti-endotoxin of typhoid bacilli can be readily prepared from animals by a process (somewhat prolonged) of immunization with the endotoxin, prepared either by grinding the bacilli, by dissolving them in bacteriolytic sera, or by allowing them to undergo aseptic autolysis. There is, however, no proof for the belief that this substance is developed during the natural process of cure, or that it is a cause of the subsequent immunity. It is quite as possible that the cessation of the febrile process is due to the destruction of the bacilli, and consequent cessation of the supply of toxin.

The effect of the true typhoid toxin is seen during the earlier stages of the disease, when the temperature is continuous. The intermittent temperature of the later stages is probably due in part to the absorption of other toxins from the ulcerated surfaces of the Peyer's patches, and in part to the liberation of endotoxins which occurs when bacilli are dissolved before being ingested.

The duration of the immunity after a natural attack of the disease is not accurately determined, but it is certainly long—perhaps several years. That due to preventive inoculation is thought to be six months at least, but here again exact figures cannot be obtained. The duration of the antibodies produced in typhoid fever varies. The agglutinins usually disappear within one or two years, but they may persist for seven or more. The bacteriolysin is thought to go sooner, but the observations on which this idea is based were mostly on the bactericidal powers of the blood, and open to fallacy.

*Diagnosis.*—Usually the Widal reaction is all that is required.

The standard that is adopted varies in different laboratories, but in the great majority of cases it will be found that the serum at the end of the first week will clump in a dilution of 1 : 30 in one hour at the body temperature, and that this is very seldom the case in health or in other diseases. The amount of agglutinin soon increases, and may reach a degree at which it will agglutinate at 1 : 1,000 or more. The naked-eye reaction as carried out by a modification of Wright's method renders it very easy to perform exact quantitative work, and it is an advantage to do so in all cases in which the agglutination does not reach 1 : 50 on the first examination, since a rise in the power of the serum is practically diagnostic. My routine method is as follows: A unit mark is made about 1 inch from the end of a rather wide Wright's pipette, and with this 9, 2, 4 and 9 units of a watery emulsion of a young agar culture of typhoid bacilli are placed in four depressions on a porcelain slab; dead cultures may also be used in the same way. A unit of serum is now sucked into the pipette and mixed with the first pool of 9 units of emulsion. One unit of this is then mixed with each of the remaining three pools, the process being carried out quickly, so as to avoid the absorption of agglutinin from the powerful serum. This gives a series of 1 : 10, 1 : 30, 1 : 50, and 1 : 100. Each pool is now sucked into a Wright's pipette sealed at the end, and incubated at 37° C. A control of emulsion without serum is also prepared and incubated. The tubes are examined at the end of one hour, and agglutination, if present, is most obvious.

The diagnosis might also be made from a determination of the opsonic index by the dilution method, or of the amount of immune body, but these processes are much more tedious.

Where a very early diagnosis is required (*i.e.*, before the appearance of the agglutination reaction) the bacilli may be sought for in the stools or blood. This was formerly difficult, but it has been greatly facilitated by the introduction of new methods involving the use of special culture media, in which the bacilli grow rapidly and form characteristic colonies. These are beyond the scope of this work, and a description of them will be found in Hewlett's "Bacteriology," second edition.

*Treatment.*—The curative treatment is not satisfactory. The use of a simple bacteriolytic serum is useless or even dangerous, for the reasons given earlier. The best hope for the future is in the use of an anti-endotoxic serum, such as was prepared by

Allan Macfadyen and others, and is now put on the market by Messrs. Burroughs and Wellcome, under the supervision of Professor Hewlett. Some promising results have already been obtained by its use, and it seems to be quite harmless. It seems only to be of advantage when used in the early stages of the disease, as is naturally the case with any serum which acts by neutralizing a soluble toxin.

Chantemesse has apparently attained a very high degree of success by the use of a serum obtained by injecting horses with a soluble toxin, prepared by cultivating the bacillus in an extract of spleen digested with pepsin and subsequently neutralized. It is very doubtful that this is the genuine toxin, since it is feeble in action and not destroyed at  $100^{\circ}\text{C}$ . The serum thus obtained is used in very small doses, and appears to act (as pointed out by Wright) rather as a vaccine than as a means of conferring passive immunity. The results, however, appear to have been excellent, but the serum is not yet obtainable commercially.

Typhoid fever has also been treated by the use of small doses of vaccine, and some promising results obtained; but the method is still on its trial.

*Preventive Treatment.*—The earliest method was that of Wright, and it has probably not been surpassed. It consists in the use of vaccines of typhoid bacilli cultivated in broth for twenty-four hours, killed at  $60^{\circ}\text{C}$ ., counted by admixture with red corpuscles, and preserved by means of lysol or carbolic acid. Two doses are given, the first being 750 to 1,000 millions, the second twice this amount, the injection being usually made deep in the subcutaneous tissue of the flank. The injections are given at intervals of one or two weeks. Local and general symptoms of some severity follow: redness and swelling at the site of inoculation, lymphangitis, etc., with fever, headache, and general malaise. These last but a short time, and no evil consequences have been recorded. It is necessary, however, for the patient to be prepared to stay in bed during the day of inoculation and the next day: the unpleasant effects may be reduced to a minimum by the simultaneous administration of chloride of calcium by the mouth (15 to 45 grains).

As a result of these injections, protective substances, and especially agglutinins, make their appearance in the blood, and may persist for several years. These may be taken as affording some test as to the degree of immunity conferred, and as to its duration.

Space does not permit us to discuss at length the evidence in favour of this method of inoculation. It has been strongly, and perhaps unfairly, opposed in certain quarters, but a careful and unprejudiced study of the statistics of the British Army in South Africa and elsewhere seems to render it quite clear that the process develops a real though not absolute protection against an attack of the disease, and is still more valuable in diminishing the mortality rate amongst those attacked. The analysis of these figures is by no means easy, but certain isolated cases are in themselves very convincing. As an example (one out of many) we may quote the Manchester Regiment, in which, out of 200 men inoculated there were 3 cases, without a death, whilst of the 517 uninoculated 23 were attacked and 3 died. In general terms we may say that the mortality from an attack fell from over 12 per cent. in the uninoculated to below 6 per cent. in the inoculated, taking the results from the whole of the Transvaal and Natal. One caution is necessary: the first result of the injection is the production of a negative phase of increased susceptibility, and injections should not be practised, if avoidable, when the patient is already exposed to the infection.

The vaccine is prepared by the Lister Institute, and can be bought ready for use.

Numerous modifications of the process have been adopted. Pfeiffer and Kolle prepare their vaccine from agar cultures. Bassenge and Rimpau do the same, but use very small doses— $\frac{1}{15}$  to  $\frac{2}{5}$  milligramme. Friedberger and Moreschi give intravenous injections of infinitesimally small doses of bacilli killed at 120° C. Wassermann's vaccine is prepared by killing a culture at 60° C., allowing it to undergo autolysis at 37° C. for five days, filtering, and desiccating *in vacuo* at 35° C. It forms a yellowish-white powder, of which the dose is 0.0017 gramme, equal to 12 milligrammes of the culture. Numerous other methods might be enumerated, but they have mostly been investigated on a small scale only, and Wright's vaccine is of proved efficacy and easy to prepare.

Antityphoid serum has often been employed, and in all probability is of value in that it affords a rapid (or practically instantaneous) means by which immunity can be produced, and eliminates the negative phase altogether. The serum is prepared from the horse, which is injected with gradually increasing doses of dead bacilli, and subsequently of living ones. It can be obtained of



great potency, as determined by its power of agglutination, which may be manifested when it is diluted a million times. But the immunity it confers (judging from laboratory experiments and analogy with other diseases) is but temporary, and the method can only be of occasional value.

The plan of injecting the serum and vaccine in combination suggested by Besredka is more promising. It is carried out as follows : A twenty-four-hour agar culture of bacilli is mixed with typhoid serum and incubated at 37° C. for twenty-four hours. The bacilli are, of course, agglutinated, and the mass is washed free from serum by repeated centrifugalizations with sterile normal saline solution. The bacilli are then suspended in this solution and heated to 60° C. for one hour. This vaccine is said to produce very rapid immunity, with practically no negative phase, and the local and general reactions it produces are but slight. It has not yet been tested on a large scale.

Simultaneous injections of serum and vaccine have also been employed by Calmette and others. The results do not appear to be as satisfactory as those obtained from the use of the vaccine alone.

### **Bacillus Coli.**

The *Bacillus coli*, in addition to its hæmolysin (colilysin) already mentioned, produces an endotoxin, on which its pathogenic action doubtless depends. Its vaccine is moderately toxic, causing severe local irritation and general febrile reaction if the initial dose be a large one. Filtrates from broth cultures are practically devoid of toxicity.

In regard to its immunity reactions *B. coli* closely resembles *B. typhosus*. Bacteriolytic and opsonic substances are developed during the course of the disease, and in both cases the opsonic index must be estimated by the dilution method, Wright's original process giving inaccurate results. There is some difference with regard to the production of the agglutination reaction, which is invariably present in typhoid infections, except in those acute cases of the disease in which the patient dies before it has time to develop. In infections with *B. coli* this is not the case, and in many of the local infections, such as cystitis, pyelitis, etc., no appreciable agglutinative properties are developed. When animals are immunized with massive doses extremely powerful clumping sera are obtained, but this is not always observed in the treatment of the human patient with vaccines.

The main difference, pathologically, between the two organisms is that, whereas the disease due to the typhoid bacillus is usually a septicæmia, localized infections (typhoid osteitis, etc.) only occur as sequelæ, the diseases due to *B. coli* are almost invariably local inflammations, blood infections, except, perhaps, as mere terminal phenomena, being rare in the extreme. These inflammatory lesions are extremely common and important, and of most diverse nature. The majority are in connection with the urinary (cystitis, pyelitis, etc.) and alimentary systems (cholangitis, cholecystitis, appendicitis, etc.). It also causes acute peritonitis, but usually in association with other bacteria. It may affect almost any part of the body, causing bronchopneumonia, otitis media, endometritis, metritis, and a host of other diseases.

The only specific treatment of any avail is the use of a vaccine. A serum has been prepared, but appears to be quite useless. The vaccine should always be prepared, if possible, from the patient's own culture, since various strains classified as *B. coli* differ slightly the one from the other. In addition to this, there are very marked differences in virulence, cultures isolated from the stools being in general less virulent than those derived from the diseased tissues. In cases in which treatment has to be prolonged it is sometimes an advantage to prepare fresh vaccines from time to time, since the organism may change its type to accommodate itself to the immune substances produced.

The initial doses should be small (10 to 40 millions), and it will be rarely found advisable to exceed 250 millions. Too large an initial dose may be badly tolerated, causing rise of temperature and a good deal of local reaction, but no permanent bad results seem to have been observed. On the other hand, it may sometimes be noted that in severe febrile cases the vaccine acts more like an antitoxin, causing a sudden drop in the temperature and a rapid amelioration of the symptoms, which may or may not be associated with a great improvement in the local lesion. This is shown in the accompanying chart, from a severe case of cystitis under the care of Mr. Burghard at King's College Hospital. As far as his general condition was concerned, he was practically well within ten days of the commencement of the treatment. The amount of pus in the urine fell to less than an eighth of its original volume, but had not disappeared entirely when he was discharged. This has been my usual experience of cystitis due

to *B. coli*—vaccine treatment cures all the symptoms and reduces the pus and bacilli present in the urine to a small fraction of its original amount, but fails to remove them entirely. Other observers have been more fortunate (Wright, Western, Norton, and others), but I believe my experience is the general one, and that complete cures are unusual. There is no doubt, however, that the treatment is the best available, the disease being notoriously resistant to simple methods of treatment. If the injections are made with opsonic control, the dilution method of

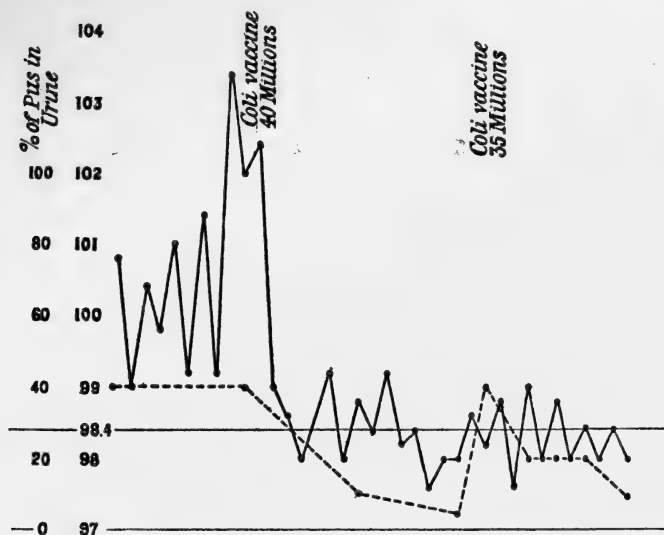


FIG. 71.—CHART FROM A SEVERE CASE OF CYSTITIS DUE TO *B. COLI*.  
The continuous line shows the temperature, the dotted line the amount of pus in the urine.

estimating the index should be used. In default of this, the injections may be given every eight to twelve days, doubling the dose until the upper limit is reached.

The treatment of inflammatory lesions of other regions (cholecystitis, persistent sinuses, etc.) appears to be more satisfactory, remarkable cures having been obtained. Butler Harris has obtained good results in endometritis, cervical catarrh, and mucous colitis. In the former disease he gives a small dose a week before and after each menstrual period.

It is in infections due to *B. coli* that some of the most striking

successes of vaccine therapy have been achieved. It has been used in some extremely severe conditions with profound toxæmia, and in no case did it render the patient's condition worse. Small doses should, of course, be used in such cases, but the severity of the disease need be no bar to the use of the remedy.

### Dysentery.

Bacillary dysentery, under which heading we may include some at least of the cases known as ulcerative colitis, asylum dysentery and infantile diarrhœa, is in its general pathology closely akin to typhoid fever. Its toxin is an endotoxin which is only set free when the bacilli undergo solution, and its cure is associated with, and perhaps due to, the development of a large amount of bacteriolysin. The main difference between the two diseases is that, whereas typhoid fever is a self-limited disease, in which the patient either dies or immunizes himself in a fairly constant period after infection, in dysentery the disease has a tendency to become chronic, the degree of immunity produced being sufficient to slow the course of the disease, but insufficient to arrest it altogether. The bacilli are in the main limited to the intestinal lesions, but the fact that they have been found in the heart-blood of a newborn fœtus whose mother was suffering from the disease leads us to believe that they may circulate in the blood; in most cases they are probably quickly destroyed, for normal serum has some bacteriolytic action.

The toxin is contained in the bodies of the organisms, which are much more poisonous than those of typhoid bacilli. It may be obtained by Macfadyen's method of grinding at the temperature of liquid air, by aseptic autolysis at 37° C., or simply by filtering old broth cultures in which some of the bacilli have undergone solution. Conradi's toxin, prepared by submitting emulsions to autolysis for forty-eight hours, was fatal to rabbits in doses of 1 c.c., causing diarrhœa, subnormal temperature, etc. According to Todd, the toxin is developed best in alkaline broth, and attains its maximum in about six weeks, after which time it begins to diminish in potency. It is more thermostable than the exotoxins, resisting heating to 70° C. for one hour, and only being destroyed slowly at the boiling-point. It is possible to obtain an anti-endotoxin, but the treatment of the animals has to be conducted with great care. It is probable also that the serum of animals immunized to the bacilli themselves contains some antitoxic

properties, especially if the bacilli are injected into the veins (Besredka). In preparing this serum it is a great advantage to follow Besredka's process, and make use of sensitized bacilli. If the unaltered bacilli are used the process presents some difficulties, especially in the case of small animals.

The serum thus prepared possesses powerful agglutinating and bacteriolytic properties, and is extremely powerful as a prophylactic agent. Kruse's serum protected a guinea-pig against a lethal dose of the bacilli in doses of  $\frac{1}{80000}$  gramme, and  $\frac{1}{10000}$  c.c. of Shiga's serum (activated with a suitable amount of complement) would sterilize 1 c.c. of a twenty-four-hour broth culture of the bacilli.

Antidysentery serum has now fully proved its value in the treatment of acute dysentery. According to Shiga, it reduces the mortality of the disease by nearly 50 per cent. Kruse claims that his serum causes a rapid diminution in the number of the stools, such as is effected by no other agent, a general improvement in the patient's condition, a shortening of the convalescence, and a diminution of the mortality. Large doses, frequently repeated, are required.

It has probably a complex action, being at the same time antitoxic and bacteriolytic, and contains opsonins and bacterio-precipitins.

In chronic cases it is of much less value, and in these forms reliance must be placed on vaccine therapy. This has been carefully studied by Captain Forster and others. In Forster's patients the mortality fell from 6.3 per cent. to 0.9 per cent., several cases of the extremely chronic type which defies all ordinary treatment for years being completely cured. He uses no opsonic control, and standardizes his vaccines by determining the minimal lethal dose; this is necessary, since the various strains differ greatly in toxicity. If the minimal lethal dose for a rabbit is about 0.4 c.c., doses of 0.1, 0.2, 0.3, and 0.4 c.c. are given at intervals of about ten days. If symptoms of an overdose are produced, the amount given at the next injection is reduced.

Prophylactic treatment by injections of killed cultures, either as they are or after autolysis or sensitization with an immune serum, or injected in conjunction with immune serum, have been used on a large scale by Shiga and others, with apparent good results as far as the case mortality is concerned, though with less obvious effect on the prevalence of the disease. This phenomenon

is readily intelligible if we regard the subsequent immunity as being a condition in which the body, having been once trained to do so, readily manufactures antibodies and other protective substances when infection occurs. Since no protective substances are present at the time, infection occurs as in a normal person, but the defensive substances are very quickly produced. Shiga's vaccine was prepared by emulsifying a twenty-four-hour agar culture of the bacillus in 5 c.c. of normal saline, heating to 60° C. for one hour, and submitting the dead bacilli to autolysis at 37° C. for two days. It is then filtered and used in doses of 0.05 to 0.5 c.c. The serum becomes strongly agglutinating and bactericidal. Other methods have been proposed.

Immunity reactions, especially that of agglutination, are of great value in the diagnosis of dysentery, and especially of the type of the bacillus present. In acute cases this is hardly necessary, since modern methods have rendered the task of isolating the bacilli from the stools an easy one. In the chronic forms this is extremely difficult, and recourse must be had to the agglutination test. The reaction is not as strong as in typhoid fever, a positive result at a dilution of 1 : 50 being diagnostic. The blood should be tested against any strains of dysentery bacilli which may be available, especially if vaccine treatment is to be used.

The method of absorption of complement has also been used.

### Cholera.

In cholera the living organisms are strictly limited to the intestinal contents, and the disease appears to be a pure intoxication, without access of living bacteria to the tissues. It is, however, probable that this is not the case, and that the vibrios enter the blood and there suffer rapid and complete bacteriolysis, their endotoxins being liberated in the process. But there is nothing that can be called a local lesion, and the disease is not a septicæmia in the ordinary sense of the word.

The toxin of cholera is a typical endotoxin. The filtrates from broth cultures are of very feeble toxicity, though they possess immunizing properties, due doubtless to some degree of autolysis which has taken place, and to the presence of free receptors. Bacilli killed with agents such as chloroform or thymol are highly toxic, especially if injected along with an immune serum, so that they can be rapidly dissolved. The endotoxin can be prepared

by aseptic autolysis, or by the freezing and grinding method of Macfadyen. In either case it is thermolabile, being largely destroyed at 60° C., so that cultures from which the toxin is to be prepared must not be killed by heat. Metchnikoff and others claim to have produced a soluble exotoxin by the use of very virulent cultures in broth: it is thermostable and not very potent. An antitoxin to it was prepared, but only very low grades of potency were obtainable. Macfadyen's toxin was much more toxic, and an anti-endotoxin of high potency was obtainable. There is no demonstrable antitoxin in the ordinary bacteriolytic serum obtained by the immunization of animals to the bodies of the bacilli, or in the serum of cholera convalescents.

Cholera presents the best example of an apparently pure bacteriolytic immunity, and presents a good example of the difficulties inherent in the explanation of this subject. The serum of an immunized animal or that of a person who has recently recovered from cholera is powerfully bacteriolytic, giving Pfeiffer's phenomenon in its earliest discovered and most marked form: it is almost the only organism which is *completely* dissolved *in vitro* under suitable conditions. Such a serum is also strongly protective, shielding animals against several times the lethal dose of living vibrios, and it seems difficult to avoid the conclusion that its preventive properties are due to its bacteriolytic action. But this is very difficult to maintain in view of the fact that the serum increases the toxic effect of dead vibrios (and under some circumstances of living ones), owing to the liberation of endotoxin. It seems rather as if the presence of bacteriolytic substances is actually harmful to the animal, allowing the organisms to set free their toxin, instead of being taken up by the phagocytes and remaining harmless. I am not aware that any opsonic experiments by the dilution method (which alone would be of value) have been carried out.

*Diagnosis.*—In dealing with cases of supposed sporadic cholera the main problem is the recognition of the vibrio isolated from the stools, usually an easy matter. The morphological and cultural characters will of course afford great help, but they take some time to work out, and more reliance is to be placed on the immunity tests, which are quicker and more conclusive. The agglutination reaction is most convenient, and can be carried out on the dejecta themselves, if the suspected organisms are present in large numbers. Some of the mucus is broken up in a little

peptone solution, and two hanging-drop preparations are made, one with the addition of normal serum in 1 : 50 dilution, the other with a 1 : 500 dilution of a powerful anticholera serum, such as can be obtained commercially. Cholera vibrios become paralyzed and agglutinated in the second specimen, not in the first. When smaller numbers are present a culture (probably impure, but with the vibrios in sufficient abundance to serve for the test) may be made by incubating peptone-water inoculated with a flake of mucus for eight to twelve hours. This is to be tested with the serum in the ordinary way, and should agglutinate at nearly the same dilution as a known cholera culture. The serum should be a powerful one, clumping at 1 : 10,000 or more.

The Pfeiffer's reaction is perhaps more conclusive, and is carried out as follows: The test immune-serum is diluted with broth or normal saline, so that 1 c.c. contains 0.001 c.c. of serum; 1 c.c. of this fluid is used to emulsify a loopful of a young agar culture of the suspected organism, and the emulsion injected intraperitoneally into a young guinea-pig. After a few minutes a little peritoneal fluid is withdrawn by means of a capillary tube, and the vibrios will be seen to have become non-motile, and to be undergoing the characteristic change into slightly refractile rounded masses. After a short time more they will be found to have disappeared altogether. A control experiment with normal serum may be made. This test is of great value, many closely allied organisms failing to react. But no test is absolutely conclusive, since a few cultures (notably the El Tor vibrio) have been found to give all or most of them, and yet have been isolated in a region in which cholera is not known to occur. The subject is not yet settled, but in the meantime the probability that any organism which reacts positively to the agglutination and Pfeiffer's tests is true cholera is enormous.

The serum of persons convalescent from cholera agglutinates the vibrios at dilutions of 1 : 100 or more for some months after the attack, a fact which may be of some value in determining the nature of a previous disease and a possible immunity to cholera.

As regards treatment, the ordinary bacteriolytic serum is quite useless, and, as far as I am aware, no potent anti-endotoxic serum has been tried. The prophylactic treatment is on a sounder footing. It was introduced by Ferran, of Barcelona, as early as



1884, very soon after the discovery of the *V. cholerae* by Koch. His results were of doubtful value, his vaccines being made of cultures of feeble virulence, and perhaps impure. The method was placed on a scientific basis by Haffkine, who showed the necessity for the use of cultures of great virulence. These are prepared by passage through guinea-pigs. A more than lethal dose of a laboratory culture is injected into the peritoneum of a guinea-pig, and the peritoneal fluid (rich in vibrios) is collected after death. This fluid is incubated in a thin layer, so as to allow of thorough aeration, for fifteen hours, and is then administered intraperitoneally into a second animal. After about twenty or thirty passages the culture will have attained its maximum virulence, the lethal dose being some  $\frac{1}{50}$  of the original. Its potency falls off in some ten days, and a few further passages are required to restore it.

The treatment is commenced by a dose of attenuated virus. This is prepared by cultivating an ordinary laboratory stock in broth at 39° C. in conditions of complete aeration. An inoculation on agar is made every day, until (after a few days) the fluid is found to be sterile. The process is now recommenced, using the last agar culture that grew, and after several generations a culture of very feeble virulence is obtained. It causes œdema, but no necrosis, when injected under the skin. The vaccines are prepared by cultivating the organisms on agar slants of definite size (10 centimetres long) for twenty-four hours, and emulsifying with 8 c.c. of broth, or 6 c.c. of 0.5 per cent. solution of carbolic acid. The dose is 1 c.c. One or two injections of the attenuated virus, followed by one of the exalted, all at intervals of three to five days, may be given, or the exalted virus only may be used. The injections cause moderate fever, headache, and general malaise, and local tenderness, swelling and enlargement of the corresponding lymph glands, all of which pass off in a few days.

This method (with various slight modifications with regard to dosage) has now been used on a very large scale in India, with strikingly good results. The immunity lasts for at least a year, and probably decidedly longer if large doses of strong vaccines are used, and, what is somewhat unusual, it manifests itself more in a reduction of the incidence of the disease than in the case-mortality. The value of the method is best seen from statistics from isolated regions in which some persons were vaccinated and others not, all living under the same conditions.

Thus, in the tea-plantations at Catchar, in 6,549 persons who were not vaccinated, there were 198 cases, with 124 deaths, whilst in 5,778 vaccinated, there were 27 cases, with 14 deaths—*i.e.*, the incidence fell from 3 to under 0.5 per cent., the case-mortality being 62 and 51 per cent. respectively. Numerous other examples might be quoted, and the value of the method is now proved to the full.

### Plague.

The plague bacillus produces a powerful endotoxin, cultures killed by heat being markedly irritating. There is some evidence that a true exotoxin may be produced, though in small amounts. Filtrates from young cultures are devoid of toxicity, whereas those from older ones may be fairly potent. The fluid portion of culture (in broth grown at 20° C. and kept well aerated) two months old was found by Markl to kill rats in doses of 0.1 c.c. This might, of course, be due to an autolysis of the bacilli, but this seems improbable from the fact that the toxicity of the filtrate is very easily destroyed by heat, whereas the endotoxin is thermostable. These filtrates have slight immunizing properties, but the plague anti-endotoxin has not been closely studied.

Immunity appears to be due to the production of bacteriolytic substances: antiplague serum, prepared by immunizing horses first with dead and then with living bacilli, is powerfully bactericidal. According to Wright, the plague bacillus is quite insensible to the bactericidal action of human blood, and recovery is due to opsonization followed by phagocytosis.

The agglutination reaction is well marked in artificially prepared immune serum, which may clump at 1:1,000 or more and may be of use in the identification of a doubtful bacillus. It is not usually marked, and may be absent in human cases of the disease, and the diagnosis is most frequently made by the identification of the bacillus in fluid from a lesion or from the blood or sputum. According to Cairns, the blood does not usually clump until the disease has been in progress for about a week. The strength of the reaction is not great, rarely rising above 1:50, and is sometimes as low as 1:3 or 1:5. The macroscopic method is advisable.

The curative treatment of the disease by specific methods resolves itself into the use of a serum, vaccines not having been tried, as far as I am aware. Several sera are prepared, but not all

have had an extensive trial. Yersin's serum is prepared at the Pasteur Institute, the process being to immunize horses for long periods—up to a year and a half—by weekly intravenous injections (which do not cause abscesses, as is the case if the injections are given subcutaneously). For the first three months or so dead bacilli are used, afterwards living ones, and a very high degree of immunity is attained. The potency of the serum is estimated by finding the smallest amount which, given twenty-four hours previously, will save a mouse from a lethal dose of living bacilli: this may be as low as 0.02 c.c. Lustig's serum is supposed to be antitoxic as well as bactericidal. It is prepared by the immunization of horses with a "toxin" prepared by dissolving plague bacilli in 1 per cent. caustic soda solution, filtering and precipitating with dilute hydrochloric acid. (This has also been suggested as a vaccine.) The precipitate is dissolved in 0.5 per cent. sodium carbonate before use.

All observers are not agreed as to the efficacy of these sera, but there is a decided preponderance of opinion in their favour. Yersin's serum is most used, and is probably of the greater value. A most important point in connection with its use is that large doses are necessary, and those observers who have not obtained good results have in some cases used quantities which were far too small. Cairns used Yersin's serum in the Glasgow epidemic, and in severe cases gave 150 to 200 c.c., part in the region draining into the affected glands and part intravenously. Choksy, as the result of large experience, urges the importance of a very early use of the remedy, and gives 60 to 100 c.c. for adults and 10 c.c. for infants, giving fresh injections of gradually diminishing amounts every twenty-four hours, until six or eight have been given in all—150 to 300 c.c. He used Lustig's serum. In any case the effect of serum is not a great one, a lowering of the case-mortality by about 10 to 20 per cent. being apparently the utmost to be hoped for at present. It appears, however, that no other treatment available is so successful.

The question of the preventive treatment is much more important. In some cases the serum may be used, and is probably most efficacious; but its effects are but transitory, and its only legitimate use is to tide the person over the time until vaccination can be performed and active immunity acquired.

Haftkine's plague prophylactic consists of a virulent broth culture of the bacillus, killed by heat and preserved by the

addition of 0.5 per cent. carbolic acid. Cultures are made in peptonized broth to which a small amount of oil is added. This floats on the surface, and serves as a point of attachment for the characteristic "stalactites." The flasks are kept at the ordinary temperature (of Bombay—about 27° C.) and shaken occasionally, to break up the stalactites. Incubation lasts five to six weeks. The vaccine is sterilized at 65° C. for one hour. The dose is 2.5 c.c. Constitutional and local symptoms of moderate severity, and lasting for a few days, are produced, but the patient is as a rule able to follow his ordinary occupation. The immunity seems to be developed quite quickly, so that there is no reason to fear any ill-effects from the injections when the patient is actually exposed to plague, and perhaps even infected. According to Bannerman, the protection is developed in twenty-four hours, and lasts about eighteen months.

Of the value of the method there can be no doubt, and statistics, both those on a large scale and those dealing with communities, some of whom are vaccinated and some not, prove clearly that the treatment lowers the likelihood of infection, and also the case-mortality. Thus, in twelve districts in the Punjab in which plague was raging in the winter of 1902-03 the following results were obtained:

	Total.	Cases.	Per Cent.	Deaths.	Per Cent.	Case-Mortality.
Uninoculated (average population of district)	639,630	49,433	7.7	29,733	4.7	60.1
Inoculated (average population of district)	186,797	3,399	1.8	814	0.4	23.9

With regard to the second group of statistics, the experience in Umarkadi Gaol may be quoted, as one out of many. Half the prisoners, selected purely by chance, were inoculated, and all lived together under exactly the same conditions. Some of each group were liberated, and of the remainder there were 127 non-inoculated, with 10 cases and 6 deaths, and 147 vaccinated, with 3 cases and no death.

The German Commission recommended the use of vaccines prepared from two-day-old agar cultures, sterilized by heat. This is more easily and quickly prepared than Haffkine's fluid.

The combined method (use of vaccine and serum) has been

recommended by Calmette, by Besredka, and by Shiga; the last-named obtained very good results by its use in an epidemic in Kobe.

### Anthrax.

The nature of the toxin of anthrax is quite unknown, although it has been the subject of much experimental investigation. No exotoxin is formed in ordinary media. If coagulable or coagulated proteids are present in the medium, they will be broken down into peptones, etc., which have some toxic action, but no true toxin is produced. Some observers have found that the filtrate from broth cultures of anthrax, though devoid of toxicity, may have some immunizing powers, a result which we should now attribute to the presence of free receptors. The only importance attaching to these facts is that they may explain the results obtained by some investigators, who obtained albumoses and other bodies of very feeble toxicity from various culture media, and considered them to be the true toxin because they served to immunize animals. And, according to Conradi, there is no evidence in favour of the existence of an endotoxin. Bacilli killed by various methods and disintegrated by Buchner's process yielded a non-toxic fluid. The clinical nature of the disease in some of its manifestations (especially pulmonary anthrax) would rather lead us to believe that a powerful toxin is produced, but of this there is not the slightest shred of experimental verification.

The process of recovery and the subsequent immunity are also very difficult to understand. Local immunity is very marked, the skin being highly resistant in comparison with the lungs, an infection of which region forms one of the most rapid and intractable diseases known in man. There are very marked differences with regard to the immunity of different animals. The fowl is highly immune, as are cold-blooded animals. The rat and dog are partially immune, whereas sheep, cattle, and the small animals of the laboratory are very susceptible.

It is especially noteworthy in the case of anthrax that the presence of bactericidal substances in the blood is no indication whatever as to the degree of immunity. The serum of the rabbit, a highly susceptible animal, has an extremely powerful bactericidal effect, whereas that of the dog and rat have but little. The classical Pfeiffer's phenomenon is not seen in the case of this bacillus, but the altered bacteria may be readily recognized from

the fact that they fail to stain by Gram's method. This change is brought about very quickly by a suitable serum, the change being often complete in ten minutes at 37° C.

There have been numerous attempts to explain the apparent anomalies of the reaction in question. Bail found that dog serum (normally a good culture medium for the anthrax bacillus) becomes highly bactericidal after the addition of a small amount of rabbit serum, even when this is only present in amount so small that it is devoid of bactericidal action *per se*. This appears to be due to the presence of immune body in the dog's blood, but no complement. If the action of the rabbit's serum is due to the presence of complement, this must be thermostable, for the effect is not annulled by heating to 50° C. Bail and Petterson found that many other sera could be reactivated with rabbit serum (man, ox, calf, pig, etc.), and that extracts of leucocytes or of organs (liver, bone-marrow) might be equally effective. Malvoz also investigated the presence of immune body by means of the Bordet-Gengou reaction (absorption of complement), and found that the amount in the serum was some index as to the degree of immunity. Thus the blood of the ox and guinea-pig contain none, as is the case with the newly-born puppy, an animal susceptible to anthrax, whereas the adult dog contains a large amount. Remy has also studied the question of the reactivation of sera of various species by complements from others, and notably that of the fowl. Thus the serum of the white rat (an immune animal) contains an immune body, for after heating to 55° C. it can be reactivated with fowl serum. On the other hand, the serum of the goat after heating cannot be rendered bactericidal in this way. He holds that there is an absolute concordance between the bactericidal power of the blood, the presence of immune body, and the resistance of the animal to infection with this organism.

Sobernheim and others have explained the susceptibility of the rabbit by supposing that the immune body has a greater affinity for the cells of the animal than for the anthrax bacillus, and is thus absorbed and rendered useless.

On the other hand, Metchnikoff holds that the immunity is entirely due to phagocytosis, and finds that the extent to which the bacteria are taken up by the leucocytes is proportional to the degree of resisting power. Anthrax bacilli (and especially the second vaccine, which forms a very good emulsion) are very

suitable objects for the study of phagocytosis. They are taken up with great rapidity, and quickly undergo solution within the leucocyte, first losing their sharp outline and power of retaining Gram's stain, and disappearing altogether in ten minutes or less. This makes the study of the opsonic index a matter of some difficulty, which can be overcome by using isolated spores in test-tube experiments. When no serum is used very few bacilli or spores are taken up, and before the discovery of the opsonins Metchnikoff noted that when rats are injected on the one side with anthrax bacilli and on the other with the same organisms mixed with blood-serum, œdema occurs only at the former place, and it is from this that generalization occurs. Sawtchenko also found that when the injection of the needle causes hæmorrhage the rat survives. The very careful and full researches of Metchnikoff on the degree of phagocytosis in susceptible and non-susceptible animals are probably sufficient to lead us to believe that the ingestion of the bacilli by the leucocytes is the all-important process in the cure of the disease, and the discovery of the opsonins supplies the missing link necessary for us to account for all the facts in a fairly satisfactory manner. We can only conclude that the bactericidal effect of the serum plays a part of comparatively small importance in combating the disease—the elaborate researches of Bail, Petterson, Sobernheim, etc., to the contrary—possibly, but by no means certainly, owing to the absence of complement.

The facts of passive immunity are not so fully explained. There are, however, some reasons for thinking that the active substance is an opsonin, perhaps a thermostable one. Thus Sclavo's serum (according to Cler) will render bacilli fit for ingestion after five hours' contact, and it does not lose its efficiency on keeping. On the other hand, the remarkably rapid improvement sometimes seen after the rise of Bandi's serum rather suggests the presence of an antitoxin.

*Diagnosis.*—This is made in all cases by the demonstration of the bacillus.

*Treatment.*—The preventive treatment is used for animals only. Pasteur's method has already been noticed: it has been largely used, and the results have, on the whole, been good. The mortality from the inoculation is about  $\frac{1}{2}$  per cent. of all cases, but in some herds the number of deaths is much higher, and serious loss is caused. The immunity is supposed to last for less

than a year, when a reinoculation is necessary. The method is not free from objections, but its use in regions of France where anthrax was very prevalent proved of enormous value, and areas in which raising cattle and sheep was rapidly becoming impossible were practically cleared of the disease. The weak point of the process is that the immunity to infection through the alimentary canal, if it exists, is extremely feeble.

To remedy the defects of Pasteur's system, Sobernheim has introduced a method of conferring mixed immunity. An anti-anthrax serum and a culture resembling Pasteur's second vaccine are injected simultaneously into different parts of the body, and no second inoculation is given. The doses are 5 to 15 c.c. of the serum and 0.5 to 1 c.c. of culture. This method of treatment is said to be free from danger, to protect against infection via the intestinal tract; it has also the advantage of requiring only a single visit. The serum is also curative.

*Curative Treatment.*—Here the use of serum is indicated. Sclavo's serum is most used in this country. It is obtained by immunizing the animals with Pasteur's vaccines, and then by giving large doses of virulent bacilli mixed with gelatin, which seems to prevent the formation of abscesses. The dose is 20 to 40 c.c., repeated in twenty-four hours if necessary, or four or five doses of 20 c.c. each: the first injection may advantageously be intravenous. It is usually followed by improvement within twenty-four hours, and often causes sweating and a rise of temperature. Sobernheim's serum is obtained by a somewhat different method, and appears to be equally efficacious. The dose recommended is 20 c.c.

The results of the use of serum in malignant pustule (which is not so dangerous a disease as was once thought, even if untreated by serum, the knife, cautery, etc.) have been very satisfactory: there do not seem to be any observations on its use in the far more serious woolsorter's disease or pulmonary anthrax. Malignant pustule is also treated by the use of very hot fomentations, the idea being to bring about the attenuation of the bacillus. There is little doubt that vaccines might be used if thought desirable in the absence of serum.

### Diphtheria.

Diphtheria presents a close approach to our idea of a disease the immunity to which is antitoxic, but it is erroneous to imagine that



the neutralization of the toxin or its destruction or elimination constitutes the whole process of cure. There is a little evidence in favour of the formation of bacteriolytic substances, though experimental evidence on this point is not unanimous. Bandi, it is true, claimed to have been able to immunize animals to the bacilli themselves, and prepared a serum which was supposed to have bactericidal properties; it has been prepared by others, and can be obtained commercially. It is supposed to be used locally, either in the form of a powder or of lozenges, and is intended to supplement the action of antitoxin. Rist, however, failed to immunize animals to the bodies of the bacilli, and though Lipstein was more successful, his serum was apparently inert as a protective agent. It contained, however, an agglutinin, and the interesting fact was noticed that it only clumps bacilli of the culture used for the injection. This is of some interest, since the Klebs-Löffler bacillus has always been looked upon as a very definite bacterial species; the toxins it produces are always neutralized by the same antitoxin, and though they may be produced in larger or smaller amounts and may contain varying proportions of proto-toxoids, etc. (on Ehrlich's theory), appear to be the same substance in all cases. These experiments would tend to show that, though the bacilli of various types agree in their metabolic products, they may differ in the constitution of their protoplasm.

The observations referred to previously, show clearly that the process of cure of the local lesion is assisted by the production of an opsonin. And there is every reason to believe that it is by phagocytosis that the bacilli are combated, bacteriolysis being very doubtful and of comparatively small importance. The cure of the disease therefore is accomplished partly by one or more of the methods discussed in Chapter VI., and partly by phagocytosis.

*Diagnosis.*—This is made by the demonstration of the bacillus. If necessary, the opsonic test might be used, and Bordet and Gengou have shown by their method of fixation of complement that "sensibilatrices" circulate in the blood. These methods are quite unnecessary. The absolute recognition of diphtheria bacillus in cultures can best be made by an application of an immunity reaction. A pure culture in broth is divided into two parts, and each injected into a guinea-pig. One of the animals receives a large dose of antitoxin, and should this remain unaffected whilst the other dies, the culture is certainly diphtheria. The

method is usually only required in cases where a healthy person contains diphtheroid bacilli in his mouth, nose, skin, etc., and considerations of public health render a determination of their exact nature necessary.

*Treatment.*—This consists in the early use of antitoxin and the treatment of the local lesion with antiseptics, and the only question of importance concerns the dosage of the former remedy. As a rule, 4,000 to 8,000 units should be given at once, and a second injection at the end of twelve or twenty-four hours; subsequent doses are given if required. Unless a case is seen very early, a part at least of the first dose may be given intravenously, and this is always advisable in severe cases not seen until the disease has been present for two or three days. Larger doses may be given, but are of doubtful advantage; a smaller amount should not be given, except perhaps in mild cases.

The sole preventive treatment in actual use consists in the use of comparatively small doses of antitoxin. The protection which is conferred is usually a strong one, but exceptions have been recorded. It lasts about a month. Essays in vaccination have been made, but not on a large scale.

### Tetanus.

The pathology of tetanus is akin to that of diphtheria in that it is a local disease with remote symptoms due to the action of a soluble exotoxin on distant structures. It differs from diphtheria mainly in two points: the bacilli are *strictly* localized to the region inoculated and the immediate neighbourhood, and the toxin, which acts entirely on the central nervous system, reaches it entirely, or almost so, by ascending the nerves from the region in which infection occurs, and not by circulating in the blood-stream. This, at least, is the usual course of events, and when, as occasionally happens, the toxin actually gains access to the blood, it seems likely that even then it does not act on the brain direct, but enters the peripheral nerves at their distal endings and then ascends them to their origin.

The diagnosis is made entirely by the recognition of the organism in the wound, no agglutination or other tests being used. If (as usually happens) the culture obtained from the wound is impure, it is divided into two parts, the one of which is injected alone, the other in conjunction with tetanus antitoxin. If no other pathogenic bacteria are present the animal that has received

the mixture will survive, whilst the other will develop tetanic symptoms and die. Even if other pathogenic bacteria are present the indications are usually clear, since spasms will commonly develop (in the animal which has received no antitoxin) before the lethal issue. It is best to use a broth culture for this test, so that there may be a good development of toxins.

The nature of the toxins of tetanus have been already mentioned. There are two, both exotoxins—the real poison, tetanospasmin, and tetanolyisin. Tetanospasmin is readily prepared by cultivation of the organism in pure culture in almost any medium under anaerobic conditions. It is even more fragile than diphtheria toxin, being rapidly rendered inert in a few days if exposed to air at ordinary temperatures. It is destroyed in eight to eighteen hours by sunlight, by a temperature of  $55^{\circ}\text{C}$ . in one and a half hours, and by exposure to agents such as alcohol, potassium permanganate, and trichloride of iodine. It can be preserved by means of dilute carbolic acid (0.6 per cent.) or chloroform without much loss. Inert solutions have in general powerful immunizing properties, the toxin being converted into toxoids, and not absolutely destroyed.

It can be prepared so as not to give the reactions for proteid, and is formed when the bacillus is grown on Uschinsky's proteid-free medium. Its potency is enormous. Thus Vaillard prepared a toxin of which the lethal dose for a guinea-pig was 0.001 c.c., containing about 0.000025 gramme of solid matter, only a small portion of which was pure toxin. Brieger and Cohn calculated that the lethal dose of an (impure) toxin for a man was 0.00023 gramme.

The effect of tetanus toxin is manifested almost solely on the central nervous system, and the post-mortem lesions are practically confined to the ganglionic cells, especially of the anterior cornua. It appears probable that there is no direct action on the nerves themselves, but the toxin, like the virus of rabies, reaches the central nervous system mainly, if not entirely, by ascending the nerves leading from the area of inoculation. According to Meyer and Ransom, toxin which gains access to the blood only affects the brain by entering the peripheral nerves via the nerve endings, especially the end-plates, but this is not universally accepted. As in the case of rabies, the richer the area of inoculation in nerves, the more powerful the action of the toxin and the shorter the period of incubation. The brain and cord are

the most susceptible regions, the peripheral nerves next, then regions with an abundant nerve supply, such as the face; and lastly, regions poorly supplied, such as the subcutaneous and peritoneal tissues. The incubation period of tetanus is thus seen to be composed of: (1) the time necessary for the production of the toxin in the tissues; (2) for its ascent of the nerves to the brain being longer, other things being equal, if infection takes place at a long distance therefrom; and (3) the latent period which elapses after the toxin has united with the ganglion cells of the central nervous system, and before the development of symptoms—*i.e.*, that in which the enzyme-like action of the zymophore group is being gradually exerted on the protoplasm. The fixation of tetanus toxin in the system is extremely rapid: in rabbits it may disappear entirely from the blood in one minute, whilst in other susceptible animals it circulates for slightly longer periods. The importance of this arises from the fact that toxin which has once entered the nerves is thereby shielded from the action of antitoxin. The dose of antitoxin necessary to save the life of an animal which has received a few lethal doses of toxin rises enormously if the injection of the former is delayed more than a few minutes.

Tetanolysin is even more fragile than tetanospasmin, being converted into toxoids in a few hours at the room temperature. It can be preserved in a dry state. The rôle which it plays in natural infections, if any, is unknown.

As regards immunity, there is but little to add to what has been discussed previously. The bacilli are not powerful parasites, being readily ingested by the leucocytes, and destroyed if the conditions are favourable for phagocytosis. In most of the cases which develop tetanus there is a contused or lacerated wound, with much killed and bruised tissues and an abundant concomitant infection with other bacteria, which still further paralyze the natural resistance of the part. These organisms may have an additional influence in securing a condition of anaerobiosis: tetanus bacilli grown in symbiosis with certain other bacteria which have powerful oxygen-absorbing properties will develop vigorously, and develop toxin in spite of the free access of air. No observations with regard to the opsonic index in tetanus appear to have been recorded. The question of immunity to tetanus toxin has been dealt with already, but we may add that in all probability much of the toxin is destroyed *in loco* by the unspecific action of the peptic enzyme formed by the leucocytes

Antitoxin is rarely, if ever, found in human patients who have survived an attack of the disease.

*Treatment.*—The main question, of course, concerns the use of antitoxin, and two general rules may be laid down: (1) It is of great value as a prophylactic agent, and (2) it is of some value in chronic tetanus—*i.e.*, the form with mild symptoms developing after a long period of incubation.

Its preventive application is indicated in the treatment of all wounds which experience has shown to be followed by tetanus—*i.e.*, lacerated and contused wounds, especially if contaminated with garden soil, road débris, etc. Gunshot wounds are especially dangerous, and tetanus is usually extremely prevalent in warfare. It is, of course, somewhat difficult to estimate precisely the value of the treatment, inasmuch as tetanus is not a common disease; but experience derived from horses, which animals are extremely prone to it, is more conclusive. In some veterinary practices it was so common as to counterindicate any operative measure, and has now been completely eradicated. The duration of the immunity conferred by a single dose is about three weeks, and in the prophylactic treatment of wounds, whether accidental or due to operation, two doses should be given, at intervals of ten to fourteen days. The prophylactic treatment of dirty wounds by means of antitoxin is now a routine method in several Continental clinics, and, as far as I am aware, there has been no case recorded in which it has been followed by the development of the disease, excepting those in which the injection has been given some days after the injury, when the toxin has already gained access to the nerves. One case (under Mr. Lenthal Cheate) from which I isolated a bacillus identical in cultural and morphological characters with that of tetanus, and in which the organisms occurred in great abundance, was treated with antitoxin at the outset, and healed without a symptom of the disease: the culture was unfortunately not tested by inoculation.

Calmette prepares a powder of dry antitetanus serum to be used as a dressing for wounds, but its use is very doubtful. Antitoxin is a good culture medium for bacteria, and unless the wound is fairly clean may decompose and become offensive. The most scrupulous antiseptic technique should be adopted, and it seems probable that the dry dressing presents no advantage over the subcutaneous administration of the serum when this is done.

The doses should be 5 to 10 c.c. for a man, and 10 to 20 c.c. for

a horse. The best method of standardization is that of Roux, who determines the amount of serum necessary to protect a guinea-pig weighing 500 grammes against ten lethal doses of toxin. The result is expressed in terms of the weight of guinea-pig protected against one lethal dose of antitoxin by 1 c.c. of serum—*e.g.*, if  $\frac{1}{10}$  c.c. protected a guinea-pig weighing 500 grammes against ten lethal doses, the potency would be 50,000. A potency of 1,000,000 is the least that should be employed.

The use of tetanus antitoxin in the developed disease is less satisfactory, a fact readily explicable now that the pathology of the disease is more fully understood. In acute tetanus it is practically worthless, though a few cures have been reported. In many cases of chronic tetanus it is without action; in a few, however, it is decidedly beneficial, each injection greatly alleviating the patient's suffering. It is always worthy of trial, but it is hardly necessary to say that the non-specific treatment should not be neglected. If the patient has a sufficient degree of immunity to resist the toxin which has already gained access to his nervous system, the antitoxin will be of value in preventing any more from doing so, inasmuch as it will neutralize it as soon as it is formed.

The doses should be large—20 c.c. or more at first, and 10 c.c. every day, or every alternate day, subsequently. The site of inoculation is of some importance. The injections may be given subcutaneously in a distant region, as in the use of diphtheria antitoxin; but, in view of the fact that it takes an appreciable time for it to be absorbed—and time is of the utmost value if the remedy is to be of any use—it seems advisable to give the first dose either in the region of the wound or intravenously.

Various methods have been proposed by which the antitoxin can be brought into closer relation with the nerve elements. The intracerebral injection has most to recommend it on theoretical grounds, and several very decided successes have been recorded in severe cases of the disease. The method is as follows: A small flap of the scalp (with its base downwards) is reflected so as to expose the skull a little to one side of the middle line, and just in front of the fronto-parietal suture. A small trephine hole is made through the skull, and an exploring needle is inserted until the lateral ventricle is reached, and cerebro-spinal fluid escapes through the needle. Ten c.c. or more of the serum are injected. This passes down the ventricular system, and bathes the

respiratory and cardiac centres at the floor of the fourth ventricle. Another method is to inject small quantities of the fluid directly into the spinal cord by means of a needle introduced between the sixth and seventh cervical vertebræ. This procedure would appear to be dangerous, but this is said not to be the case. Lastly, the simplest method of all is to perform lumbar puncture, draw off some of the cerebro-spinal fluid, and replace it with serum, just as in the process of spinal anæsthesia.

Ransom and Meyer have advocated the direct application of antitoxin to the nerves supplying the region in which the wound is situated, the idea being, of course, to intercept any further access of toxin to the brain and cord. The nerves are exposed by operation as near to their origin as possible, and infiltrated with serum by means of a hypodermic syringe.

Analogy with other diseases would fully justify the use of vaccine in chronic tetanus. Its preparation would present some difficulties, owing to the heat-resisting power of the spores.

### Syphilis.

Little is known definitely concerning the mode of cure or of the type of immunity of syphilis. It used to be regarded as one of the diseases which are followed by practically complete immunity of long duration, but Neisser has brought forward some evidence for thinking that this is not the case, and that it only lasts as long as the disease itself—*i.e.*, as soon as it is *completely* eradicated the patient is again susceptible. Nothing is known as to the toxins of syphilis, and, as regards the method of cure, the only point worth mentioning is the fact that spirochætes which have been ingested by the leucocytes can be made out occasionally. They stain badly, and are doubtless on the way to complete absorption. The fact that the organism cannot be obtained in pure culture renders researches with regard to the opsonic and bacteriolytic action of the serum very difficult. Indirect researches by means of the deviation of complement—constituting the Wassermann reaction, a special method of application of the Bordet-Gengou reaction—have led to results of great interest which have recently attracted much attention.

The first necessity was, of course, the preparation of an antigen, and for this purpose Wassermann made use of the internal organs of a syphilitic foetus, which were swarming with spirochætes. In

its main outlines the technique is exactly the same as that already described. The serum to be tested is heated, to remove complement, and diluted with sterile normal saline solution. A dilution of 1 : 20 or 1 : 40 is generally correct, but the point may be determined by preliminary tests with a known syphilitic serum ; and in any case it is an advantage to perform a series of tests with different dilutions, so that a rough idea of the amount of antibody present in the serum may be obtained. This is mixed with an extract of the syphilitic organ (antigen), and some fresh guinea-pig serum (complement) added. The proportions may be 1 c.c. of diluted serum, 0·1 or 0·2 c.c. of organ extract, and 0·2 c.c. of fresh serum. The whole is incubated for one hour, at the end of which time all the complement will be removed from the fluid if syphilitic antibody is present. Next, corpuscles (*e.g.*, of a sheep or pigeon) are added, together with heated serum from a rabbit which has been injected with the corpuscles in question ; or the corpuscles may previously be sensitized with the inactivated serum, washed, and then added. The whole mixture is then incubated for two hours, with occasional stirring or shaking, and kept some hours in the ice-chest. A positive reaction is shown by the absence of hæmolysis. Control tests are also advisable—*e.g.*, the corpuscles must be completely dissolved by the heated immune serum and the guinea-pig's serum if the other two ingredients are not added, and there should be no hæmolysis if all the substances except the guinea-pig's serum are used.

Ledingham and Hartoch have shown independently that opsonin is absorbed as well as complement, and this fact may be used as a test of the presence of the reaction. In this case the first part of the test is performed as before, and the fluid used as the serum in an opsonin estimation, using staphylococci or any other organism, and using as a control guinea-pig serum diluted with normal saline to the same extent as it was in the mixture of organ extract, human serum, and guinea-pig serum. In a positive reaction the phagocytic index in the first preparation will be much below that in the second ; in a negative one they will be equal.

The exact value of the test is not yet quite definitely settled. It is very rarely present in health, and not common in diseases other than syphilis ; but it does occur, especially in diseases which (like syphilis) are due to animal parasites, such as malaria or trypanosomiasis, and is not uncommon in leprosy and scarlet



fever. It is rarer in other diseases, but isolated examples have been met with in systematic investigations in a great many maladies; but here it is the exception, whereas in syphilis it is the rule. In primary and secondary cases it occurs in 90 per cent. or more, and is present in the majority of patients suffering from tertiary syphilis and "metasyphilitic" affections. It is very frequently found in the cerebro-spinal fluid of general paralytics (80 per cent., 90 per cent., or more), even when it is absent from the blood. It is not so common in tabes, and is extremely rare (if it ever occurs) in the cerebro-spinal fluid in non-syphilitic diseases, with the curious exception of scarlet fever, in which it is almost constant.

So far there is no theoretical difficulty in the interpretation of the phenomenon, but a new fact discovered by Landsteiner, Müller and Pötzl seems to show that the reaction is of a nature entirely different from the ordinary Bordet-Gengou phenomenon. They found that an alcoholic extract of a normal organ (*e.g.*, of a guinea-pig's heart muscle) might be used instead of a tissue rich in spirochætes; and further researches have shown that the lipid substances isolated therefrom, or even comparatively simple substances, as lecithin and taurocholate and glycocholate of soda (Levaditi and Yamanouchi) give the reaction, although apparently not so frequently, as when an extract from a syphilitic organ is used. The "antigen" is soluble in hot alcohol, and this fact alone removes it from the group of *true* antigens, which, as we have seen, are apparently all proteid in nature. According to Levaditi, the substance occurring in the blood or cerebro-spinal fluid is not an antibody at all, but either lipid substances or salts, or the two in combination, and they are set free when tissues are broken down in a certain way, which occurs most frequently in syphilis, but may take place in other diseases. Under ordinary circumstances they are present in a colloid state, but form a precipitate with the lecithin and allied substances extracted from normal organs by hot alcohol, and to this precipitate the complement attaches itself. According to Porges, the serum of syphilitics has the power of precipitating an emulsion of lecithin (0.5 gramme, shaken up with 0.5 per cent. solution of carbolic acid in normal saline) when mixed therewith in equal parts. This he proposed as a test for syphilis, and Nobl and Arzt found it successful in 80 per cent. of cases. Subsequently, Porges replaced the lecithin (which as usually bought is not constant in composition) by a recently prepared 1 per cent. solution

of glycocholate of soda. A mixture of this with an equal amount of serum is incubated for five hours, and observed after it has stood sixteen to twenty hours at the room temperature. The precipitate is specially obvious near the surface.

Fornet, Schereschewsky, Eisenzimmer, and Rosenfeld find that the sera of syphilitics in the early stages of the disease contain a precipitogen which forms an insoluble compound with a substance or precipitin present in the serum of tabetics or general paralytics. When the one is floated on the other, a characteristic ring appears at the area of contact. They say that normal serum rarely contains the precipitin, but not the precipitogen. What relation this has to any immunity reaction is unknown.

### Rabies.

The actual causal agent of rabies is not yet definitely ascertained. The peculiar structures known as the corpuscles of Negri which occur in the brain, and especially in the hippocampus major, of rabid animals appear to be quite characteristic of the condition, and may possibly be the actual parasite, although this is not yet universally accepted. It seems, however, fairly certain that their recognition constitutes a sufficient proof of the presence of the disease; and this is of great importance in view of the necessity for the early commencement of the treatment, which is entirely preventive, and not curative. If the dog by which the patient has been bitten is forthcoming, the corpuscles of Negri can be demonstrated in a short time by simple methods, and the need for Pasteur's treatment ascertained; apart from this the only method is by animal inoculation, an emulsion of brain substance being injected into the brains of rabbits after trephining.

Rabies presents one of the most striking examples of local immunity; the action of the virus is manifested almost entirely on the central nervous system, and in whatever part of the body the inoculation is made the effects are only caused when it has reached the brain and spinal cord; and in doing so it does not gain access to the blood, but ascends the peripheral nerves. Hence the central nervous system is extremely susceptible to injection, and the other tissues in proportion to their richness in nerves. Subcutaneous (unless into a region like the paw), intravenous, or intraperitoneal injections only convey the disease if a large amount of extremely potent virus is used. Hence it seems

reasonable to suppose that there is a fair amount of immunity inherent in all the tissues except in the nervous structures, and that the living virus deposited elsewhere may be entirely destroyed by bacteriolysis or phagocytosis.

We have already glanced briefly at Pasteur's earlier work on antirabic inoculations, and the method by which immunity is produced. Numerous modifications of the process have been introduced since Pasteur's time. Thus Högyes of Budapest makes use of fully virulent cords, but given in extremely small doses; and there is some reason to think that his process does not really differ from that of Pasteur, and that in drying the cords the virus is gradually destroyed and not really attenuated, so that a dose of a fourteen-day cord really contains a small trace of virus of full virulence. A true vaccine—*i.e.*, a virus of mitigated virulence—can be obtained by passage through monkeys or birds. Further, though the fixed virus is so potent for rabbits, it is quite possible that its virulence for man is slight or nil. Nitsch was so sure of this that he injected 4 to 5 milligrammes of the fresh cord subcutaneously unto himself (in the abdominal region, a part comparatively poor in nerves) without evil results.

Another method, introduced by Marie, consists in the use of injections of a mixture of virus and serum from an immunized animal. This serum is prepared in a variety of ways, the simplest being to give the virus intravenously. The animal usually employed is the sheep, and the injection consists of rabid brains, heated up into a fine emulsion with normal saline solution, and filtered through linen. The serum prepared from animals treated in this way possesses powerful antirabic properties: when mixed with a potent virus it removes entirely all harmful properties, so that it is quite innocuous even on intracerebral injection. It can be titrated against an emulsion of fixed virus of definite strength, and by appropriate treatment a very potent serum can be obtained. It is apparently quite useless in the treatment of the developed disease or of an infected animal, even before the development of symptoms. If it is mixed in excess with fixed virus and injected into animals these do not develop rabies; on the other hand, but little immunity is produced, and this is supposed to be due to the fact that the virus is so quickly absorbed that it does not act as an antigen. But if the mixture be allowed to stand for some time, and the virus then recovered by centrifugalization and washing with normal saline solution, the clot thus obtained has

powerful immunizing properties. In the preventive treatment of rabies on Marie's system the fresh fixed virus, made into a fine emulsion with normal saline solution, is *partially* neutralized with immune serum, and a dose of 6 c.c. (2 c.c. of 1 : 10 emulsion of virus and 4 c.c. of serum) is given in two places under the skin of the abdomen. This is done for four days, and then injections of dried cord, beginning at that of the sixth day, are commenced.

Other methods involve the use of heat, of chemical methods (*e.g.*, partial digestion with gastric juice, as practised by Centanni), in order to bring about attenuation or partial destruction of the virus.

Whatever the method, it appears necessary that the patient should undergo a course of active immunization, various causes (*e.g.*, the long incubation period and the localization of the virus in the nerves) rendering passive immunity an unsafe method of protection.

Of the value of the process there cannot be the slightest doubt.

The incidence of hydrophobia after the bite of a rabid animal is variously estimated, the figures usually given being about 15 per cent. in the case of dog-bites, and 40 to 80 per cent. in bites from wolves. The probability of the patient's developing the disease depends on the severity of the bite, its position (*i.e.*, whether in regions rich in nerves or the reverse), and on whether the bite is through the clothing, so that some of the virus is wiped from the teeth. In the twenty-two years (down to 1907 inclusive, the last year of which the figures are available), 30,359 patients have been treated at the Pasteur Institute in Paris, with 126 deaths—a death-rate of 0.31 per cent. (The patients dying within fifteen days of the commencement of the treatment—a small number—are excluded from the figures, since in them the disease was too far advanced for a preventive treatment to be of value.)

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[A complete bibliography of the subject being obviously impossible in any reasonable space, an attempt has been made to include important articles, and especially those referred to in the text, and articles dealing with the subjects in a complete manner, especially those with a good account of the literature.]

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